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Application of Fourier Transform Infrared Spectroscopy in the Analysis of Edible Fats and Oils

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Short title:

FTIR ANALYSIS OF EDIBLE FATS AND OILS

ABSTRACT

The application of Fourier transform infrared (FTIR) spectroscopy in the assessment of oil quality and stability and the determination of the degree and type (*cis* or *trans*) of unsaturation of fats and oils was investigated. FTIR spectroscopy was shown to provide a rapid means of monitoring changes in oils undergoing oxidation or subjected to thermal stress. Absorption bands associated with common primary and secondary oxidation products were identified by relating them to those of spectroscopically representative reference compounds, and a quantitative approach based on the use of oils spiked with these reference compounds as calibration standards was proposed. A sample-handling accessory based on a heated 25-μm transmission flow cell and heated input and output lines was developed to facilitate the rapid analysis of oils and premelted fats in their neat form. Using this system, an FTIR edible oil analysis package was developed to simultaneously analyze for *trans* content, *cis* content, iodine value (IV), and saponification number (SN) of neat fats and oils, using partial-least-squares (PLS) calibrations based on pure triglycerides. An automated transmission-based peak height method for isolated *trans* isomer determination using the characteristic *trans* absorption band at 967 cm^{-1} in the spectrum of a neat fat or oil, ratioed against the spectrum of a *trans*-free oil, was also developed. A subsequent validation study involving the analysis of more than 100 oil samples demonstrated concurrence between the *trans* data obtained by the PLS and peak height FTIR methods as well as between IV results obtained by FTIR analysis and gas chromatography. In addition, the internal consistency of the IV, *cis*, and *trans* FTIR predictions provided strong experimental evidence that the FTIR edible oil analysis package measures all three parameters accurately. A PLS-based IV/*trans* method was developed for a heated single-bounce horizontal attenuated total reflectance (SB-HATR) sample-handling accessory and shown to be similar in accuracy and reproducibility to the transmission methods developed. The research carried out demonstrates that the degree of unsaturation (IV) and type (*cis* or *trans*) of unsaturation of fats and oils can be quantified reproducibly and accurately by FTIR spectroscopy and that the chemical changes taking place as an oil undergoes oxidation can be monitored effectively. These results indicate that with appropriate integration of instrumentation, software, and sample-handling accessories, FTIR spectroscopy has the potential to become an important analytical tool in the fats and oils industry.

RÉSUMÉ

La pertinence d'une utilisation de la spectroscopie à infrarouge à transformation de Fourier (FTIR) en référence avec l'évaluation de la qualité et de la stabilité des huiles ainsi que pour la détermination du degré et du genre d'insaturation (*cis* ou *trans*) des gras et des huiles a été étudiée. Il a été démontré que la spectroscopie FTIR constitue un moyen rapide et efficace de mesure en ce qui a trait à la transformation des huiles tant au niveau de leur oxydation que de leur comportement thermique. Les fréquences auxquelles absorbent les produits primaires et secondaires se retrouvant couramment lors d'un processus d'oxydation ont été déterminées par comparaison aux spectres infrarouges de produits de référence. Ensuite, une méthode quantitative basée sur des échantillons d'huile avec différentes quantités ajoutées des produits de référence a été suggérée. Un accessoire utilisant une cellule de transmission chauffée dont les lignes d'introduction et d'éjection sont aussi chauffées a été mise au point afin de faciliter une analyse rapide des huiles ainsi que des gras pré-fondus tout en ne nécessitant aucun prétraitement. Des calibrations utilisant la méthode des moindres carrés partiels (PLS) pour prédire la teneur en *trans*, la teneur en *cis*, l'indice d'iode (IV), et la valeur de saponification des huiles et des gras non traités ont été développées. Parallèlement, une méthode automatisée prédisant uniquement la teneur en *trans* et se basant sur le calcul de la hauteur du pic à une fréquence de 967 cm^{-1} (caractéristique des produits *trans*), après soustraction d'un spectre d'huile ne contenant pas de produits *trans* du spectre de l'huile analysée, a été développée. Par la suite, une étude ayant pour but de procéder à la validation des deux méthodes et impliquant l'analyse de plus de 100 échantillons d'huile a démontré que d'une part, les prédictions obtenues pour la teneur en *trans* par PLS et par l'approche de la hauteur du pic coïncidaient, et d'autre part, les prédictions de IV obtenues par FTIR concordaient avec ceux de la chromatographie en phase gazeuse. De plus, la consistance entre les valeurs obtenues pour la teneur en *cis*, la teneur en *trans*, et la IV confirmait que l'analyse des huiles par FTIR produit des résultats d'une grande précision pour tous les paramètres étudiés. Une méthode pour l'analyse de IV et de la teneur en *trans* basée sur l'approche PLS pour un accessoire de réfléctance totale atténuée n'ayant qu'un point de réflexion a aussi été développé. Cette dernière approche a donné des résultats comparables, en terme de précision et de reproductibilité, aux méthodes développées pour l'accessoire de transmission. La recherche entreprise démontre que le degré (VI) et le genre (*trans* ou *cis*) d'insaturation des huiles et des gras peuvent être quantifiés avec précision et de façon reproductible par la spectroscopie FTIR. De plus, les changements chimiques se produisant durant l'oxydation des huiles peuvent être suivis de façon efficace. Les résultats obtenus indiquent donc que grâce au support d'une combinaison judicieusement sélectionnée d'instrumentation, d'accessoires et d'applications informatiques, la spectroscopie FTIR a un potentiel indéniable en tant qu'outil d'analyse dans le secteur industriel des huiles et des gras.

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Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" and must be bound together as an integral part of the thesis.

The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges between the different papers are mandatory.

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As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

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When previously published copyright material is presented in a thesis, the candidate must obtain, if necessary, signed waivers from the co-authors and publishers and submit these to the Thesis Office with the final deposition.

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I would like to express my deepest gratitude to Professor F. R. van de Voort for the tremendous amount of guidance and encouragement that he has given me throughout the course of this work. His exceptional qualities as a research supervisor have made this thesis possible. I would also like to thank Professor Ashraf A. Ismail for his help, support, and valued advice, as well as for originally bringing me into the McGill IR Group. I would like to express my appreciation to Mrs. Lise Stiebel and Mrs. Barbara Laplaine in the departmental office for their helpfulness and kindness and to my colleagues in the McGill IR Group for helping to make the years that I have spent with the group stimulating and rewarding.

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CONTRIBUTIONS OF AUTHORS

Chapters 4-7 of this thesis are the text of the published papers listed below. The present author was responsible for the concepts, design of experiments, experimental work, and manuscript preparation. Drs. Van de Voort and Ismail are thesis supervisor and co-director of the McGill IR Group, respectively, and had direct advisory input into the work as it progressed. Mr. G. Emo provided technical support for the experiments described in Chapter 4, and Dr. P. Maes provided the samples and gas chromatographic data for the studies reported in Chapter 6 and contributed his expertise in the interpretation of the results.

List of the publications reproduced in the thesis:

F. R. van de Voort, A. A. Ismail, J. Sedman, and G. Emo, Monitoring the oxidation of edible oils by Fourier transform infrared spectroscopy, *J. Am. Oil Chem. Soc.* 71:243-253 (1994).

F. R. van de Voort, A. A. Ismail, and J. Sedman, A rapid, automated method for the determination of *cis* and *trans* content of fats and oils by Fourier transform infrared spectroscopy, *J. Am. Oil Chem. Soc.* 72:873-880 (1995).

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J. Sedman, F.R. van de Voort, and A. A. Ismail, Simultaneous determination of iodine value and *trans* content of fats and oils by SB-HATR Fourier transform infrared spectroscopy, *J. Am. Oil Chem. Soc* (in press).

Portions of Chapters 2 and 8 are drawn from contributions made by the present author to the following publication:

J. Sedman, F.R. van de Voort, and A.A. Ismail, Application of Fourier transform infrared spectroscopy in edible oil analysis, in *New Techniques and Applications in Lipid Analysis*, edited by R. E. McDonald and M. M. Mossoba, AOCS Press, Champaign, Illinois, 1997, pp. 283-324.

CONTRIBUTIONS TO KNOWLEDGE

1. **Illustrated the utility of the technique of differential spectroscopy as a means of monitoring oxidative changes in edible oils by Fourier transform infrared (FTIR) spectroscopy.**

   The spectral changes taking place during oil oxidation were shown to be clearly discernible by ratioing the spectra recorded during oil oxidation monitoring against the spectrum recorded from the fresh oil prior to oxidative changes. The changes detected by differential spectroscopy were interpreted by comparison to the spectra of reference compounds representative of primary and secondary oxidation products.

2. **Demonstrated the utility of a calibration approach based on the use of pure triglycerides as calibration standards and partial-least-squares regression (PLS) for the development of an FTIR method for the prediction of the *cis* and *trans* content of fats and oils.**

   The validity of this universal calibration approach was established through extensive validation studies with processed fats and oils.

3. **Developed a new transmission-based peak height method for the determination of *trans* content that takes advantage of the spectral ratioing capability of FTIR spectrometers.**

   The method developed simplifies IR *trans* determinations by allowing for analysis of fats and oils without the need for saponification and methylation or dissolution in a solvent. The method was shown to match the accuracy of the official method of the American Oil Chemists' Society (AOCS) through validation studies conducted with AOCS Smalley check samples.

4. **Developed a method for the determination of iodine value by single-bounce horizontal attenuated total reflectance (SB-HATR) that can be paired with the new AOCS FTIR method for the determination of isolated *trans* isomers.**

   Implementation of this method in quality control laboratories would provide a rapid, simple, and cost-effective alternative to gas chromatographic (GC) analysis in situations in which only IV and *trans* data are required.

5. **Provided experimental evidence that both PLS and the spectral ratioing approach successfully compensate for the triglyceride interferences that have traditionally limited the accuracy of IR *trans* analysis.**

   Over 100 samples of partially hydrogenated oil were analyzed by both the PLS-based and peak height *trans* methods. The excellent agreement obtained between the two sets of predictions demonstrated the efficacy of both calibration approaches.

6. **Developed the first SB-HATR method for the analysis of fats and oils based on a multivariate calibration approach.**

   Demonstrated that the SB-HATR sample-handling technique provides adequate spectral reproducibility to meet the stringent requirements imposed by multivariate calibration techniques.

7. **Corroborated preliminary indications in the literature that the recently approved AOCS method for GC determination of *trans* fatty acid content (Ce 1c-89) provides results that match those obtained by FTIR spectroscopy.**

   The concurrence obtained between the FTIR-predicted *trans* values and GC *trans* data for a set to 10 hydrogenated oils indicates that both the IR and GC methods measure *trans* content accurately. This represents a substantial advance in fats and oils analysis in that the *trans* values obtained by the traditional GC and IR methods differed by up to 30%.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
AOM	Active oxygen method
ATR	Attenuated total reflectance
AV	Anisidine value
CHD	Coronary heart disease
CV	Coefficient of variation
DSC	Differential scanning calorimetry
FAME	Fatty acid methyl ester
FDA	Food and Drug Administration
FFA	Free fatty acid
FT	Fourier transform
FTIR	Fourier transform infrared
GC	Gas chromatography
HATR	Horizontal attenuated total reflectance
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
IV	Iodine value
LDL	Low-density lipoprotein
MD_a	Mean difference for accuracy
MD_r	Mean difference for reproducibility
NMR	Nuclear magnetic resonance
OSI	Oil stability index
PCR	Principal component regression
PHVO	Partially hydrogenated vegetable oil
PLS	Partial least squares
PRESS	Predicted residual error sum of squares

PV	Peroxide value
QC	Quality control
RBD	Refined, bleached, deodorized
SB-HATR	Single-bounce horizontal attenuated total reflectance
SD	Standard deviation
SDD_a	Standard deviation of the differences for accuracy
SDD_r	Standard deviation of the differences for reproducibility
SE	Standard error
SFC	Solid fat content
SFI	Solid fat index
S/N	Signal-to-noise ratio
SN	Saponification number
TLC	Thin-layer chromatography
TPP	Triphenylphosphine
TPPO	Triphenylphosphine oxide
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Infrared (IR) spectroscopy is among the most powerful techniques for qualitative analysis because of the detailed information about chemical composition and molecular structure that is contained in the IR spectrum of a substance. The absorption bands in the IR spectrum of a sample represent the excitation of vibrational modes of the molecules in the sample and thus are associated with the various chemical bonds and functional groups present in the molecules. Thus, the IR spectrum of a compound is one of its most characteristic physical properties and can be regarded as its "fingerprint." IR spectroscopy is also a powerful tool for quantitative analysis as the amount of infrared energy absorbed by a compound is proportional to its concentration. However, its utility as a technique for quantitative analysis has traditionally been fairly restricted, in large part because of the limitations of the traditional dispersive spectrometers, the lack of the computational capabilities required for the quantitative analysis of multicomponent systems, and difficulties in sample handling. Thus, although IR spectroscopy found some applications as a quantitative analysis tool in the 1940s and 1950s, it was largely eclipsed in the 1960s and 1970s by other techniques, particularly gas chromatography and nuclear magnetic resonance spectroscopy.

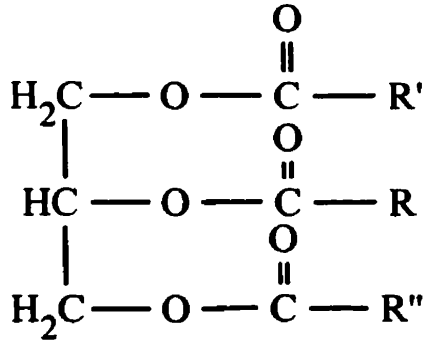
During the past three decades, however, IR quantitative analysis has been revitalized, primarily owing to the development of Fourier transform infrared (FTIR) spectroscopy, coupled with advances in software and chemometrics, and reductions in the cost of FTIR instrumentation during the past ten years have led to interest in the potential

uses of FTIR spectrometers as dedicated analyzers for specific industrial quality control and process monitoring applications (1-4). The potential benefits of quantitative FTIR spectroscopy for such industrial applications include rapid and reproducible analyses, amenability to automation, a reduction in chemical waste and analytical costs, and the possibilities for at-line and, with the use of optical fibers, on-line analysis.

The research on the analysis of fats and oils by FTIR spectroscopy presented in this thesis was undertaken in this context. The fats and oils industry continues to rely largely on wet chemical and chromatographic methods for its routine analytical requirements. However, there is growing interest within the industry in replacing these well-established methods with automated instrumental methods in order to improve efficiency and address environmental concerns about the use of large volumes of solvents and reagents in quality control laboratories (5). Edible fats and oils are ideal candidates for FTIR analysis, because the FTIR spectra of oils and melted fats can be recorded from samples in their neat form, without any sample preparation (6). However, there is little familiarity with FTIR spectroscopy in the edible oil industry, and most quality control laboratories are thus not in a position to develop and implement FTIR methods. Accordingly, widespread application of FTIR spectroscopy for the analysis of edible fats and oils will likely only occur if the industry is provided with practical methods that can be directly implemented and do not impose new problems or constraints or an extensive learning curve (6). The overall aim of the work presented in this thesis was to investigate the means by which this latter goal can be achieved.

1.1. GENERAL OVERVIEW OF FATS AND OILS ANALYSIS

Edible fats and oils are obtained from a wide variety of plant and animal sources. Regardless of their origin, these fats and oils are composed predominantly of triglycerides (>95%), which are triesters of fatty acids and glycerol:



However, fats and oils from different sources differ in their triglyceride composition, and these differences give rise to wide variations in physical and functional properties. Important determinants of these properties are the lengths of the fatty acid chains, ranging from 4 to 22 carbons, their distribution on the glycerol backbone, and their degree of unsaturation. For example, the melting point of a trig'yceride, which is one of the most important physical properties in relation to functionality, decreases with a decrease in chain length or an increase in the degree of unsaturation. Another example of a property of fats and oils that is highly dependent on their degree of unsaturation is oxidative stability. The reaction between unsaturated lipids and atmospheric oxygen under ambient conditions, termed lipid autoxidation, is a leading cause of deterioration of fats and oils, as well as of any lipid-containing food. The susceptibility of triglycerides to autoxidation increases, and thus their oxidative stability decreases, as the number of double bonds in the fatty acid chains increases. As such, among the wet chemical methods currently

employed by the fats and oils industry, one of the most widely used is the determination of iodine value (IV), which measures total unsaturation via the reaction of halogens with double bonds.

Most unsaturated bonds in naturally occurring fats and oils are in the *cis* configuration. *Trans* double bonds are present in fats from ruminants, owing to *cis* → *trans* isomerization during biohydrogenation of dietary lipids by bacteria in the rumen. For example, the level of *trans* isomers in bovine fat ranges from 2 to 7% of total fatty acids, depending on breed, climate, and feed (7). *Trans* double bonds are also naturally present in some marine fats and in the conjugated polyene acids that occur in a few seed oils, such as β-eleostearic acid (18:9*t*,11*t*,13*t*), which is the major fatty acid in Chinese tung oil (8). However, the presence of *trans* double bonds in vegetable oils and the fats produced from them is primarily due to *cis* → *trans* isomerization during industrial processing, and these processed fats and oils are the major sources of *trans* fatty acids in the North American diet (9, 10). As will be discussed in more detail later in this section, the levels of *trans* fatty acids in the diet have increasingly become of nutritional concern in recent years, in relation to cardiovascular disease.

The production of commercial fats and oils involves a number of processes, including (1) extraction of oils/fats from their natural sources (e.g., oilseeds, animal tissues) by pressing, rendering, or solvent extraction; (2) refining, in order to remove unwanted components, primarily free fatty acids, phosphatides, and mucilaginous material; (3) bleaching, in order to remove pigments and other colored impurities; and (4) deodorization, in order to remove traces of constituents that give rise to odor and flavor.

Apart from these separation/purification processes, the processing of oils frequently entails an additional step in which catalytic hydrogenation is performed to modify the properties of the oil by altering its triglyceride composition. This process involves the addition of hydrogen across double bonds in the presence of a metal catalyst, usually nickel, leading to a decrease in unsaturation and hence an increase in melting point and in oxidative stability. Usually, catalytic hydrogenation processes are carried out under conditions leading to partial hydrogenation rather than total hydrogenation of all double bonds; under such conditions, there is extensive positional and geometric (i.e., *cis* → *trans*) isomerization of the remaining double bonds. *Cis* → *trans* isomerization also contributes to the hardening effect, as the melting points of *trans* fatty acids are higher than those of the corresponding *cis* isomers but lower than those of their saturated homologues. Catalytic hydrogenation is widely employed to convert oils to plastic fats for use in margarines and shortenings and to increase the oxidative stability of highly unsaturated oils, although in recent years the nutritional concerns about *trans* fatty acids have led to increased use of interesterification for the production of plastic fats without the formation of *trans* isomers. Interesterification involves the randomization of fatty acid chains among the various triglycerides present in a single oil or two oils mixed together and thus entails changes in fatty acid distribution, leading to alterations in melting behavior. Interesterification of a liquid vegetable oil with a fully hydrogenated fat yields a product having characteristics similar to those of a partially hydrogenated oil but containing no *trans* isomers (11). In contrast, the levels of *trans* fat in margarines formulated with partially hydrogenated oils can exceed 45% of total fat because of

extensive *cis* → *trans* isomerization during catalytic hydrogenation (10). Food products prepared with or fried in partially hydrogenated vegetable oil (PHVO) shortenings are also important contributors of dietary *trans* fatty acids (12).

Although *trans* isomers in processed fats and oils are predominantly the result of isomerization during catalytic hydrogenation, *cis* → *trans* isomerization also occurs during deodorization at high temperatures (13). In the latter case, the isomers formed are predominantly di- and trienoic mono-*trans* fatty acids owing to the higher thermal reactivity and oxidative instability of polyunsaturated fatty acids. Although dienoic mono-*trans* fatty acids are also formed to some extent during catalytic hydrogenation, together with small amounts of di-*trans* fatty acids, the major *trans* isomers in hydrogenated oils are monoenes (14).

The fats and oils industry routinely employs a number of "official" analytical methods of the American Oil Chemists' Society (AOCS) and the Association of Official Analytical Chemists (AOAC) for quality control of its products and as a basis for setting product specifications. These include methods for the determination of acid value (a measure of free fatty acid content), iodine value, moisture, unsaponifiable matter, insoluble impurities, ash, and peroxide value (an indicator of oxidative status) (15). Analyses are also performed for process control; in particular, catalytic hydrogenation is a highly variable process and the product distribution obtained depends on a large number of parameters, making it essential to monitor the process using one or more analytical techniques, such as the determination of iodine value or solid fat index/solid fat content (16). Another important analysis in the characterization of partially hydrogenated fats and

oils is the determination of *trans* content, as the extent of *cis* → *trans* isomerization that occurs during catalytic hydrogenation affects the functional properties of the product .

A variety of analyses are also performed to assess oil quality and stability, that is, to evaluate certain characteristics that govern the acceptability of the oil for its intended uses, and ultimately the acceptability of the oil or foods prepared with it to the consumer, and to establish the resistance of the oil to change over time (17). The acceptability of an oil is determined by such characteristics as appearance (e.g., clarity), color, flavor, and odor, the desired characteristics for a high-quality oil being dependent on the oil type. In addition, various other characteristics are important for particular intended uses of the oil (e.g., texture in the case of oils hardened for use as shortenings and in the preparation of margarines). Similarly, oil stability is defined in terms of the intended uses of the oil, as the conditions that an oil is required to withstand during deep-fat frying, for instance, differ substantially from those pertinent in the evaluation of a salad oil.

Apart from analyses performed for the traditional purposes of quality control and process monitoring, in recent years additional analyses have become required for compliance with new government regulations. In particular, the adoption in the United States of the Nutrition Labeling and Education Act in 1990 led to the requirement for declarations of the amounts of total fat, saturated fat, monounsaturated fat, and polyunsaturated fat on the labels of food products (18). In addition, in late 1999, the U.S. Food and Drug Administration (FDA) submitted a proposal to amend the nutrition labeling regulations to require the inclusion of *trans* fatty acids in the amount stated for saturated fat, together with a footnote on the food label reporting the *trans* fatty acid

content (19). This proposal stems from the concerns that have arisen in the past decade about the nutritional implications of dietary *trans* fatty acids, following the publication in 1990 of findings that *trans* fatty acids elevate total plasma cholesterol and low-density lipoprotein (LDL) cholesterol and lower high-density lipoprotein (HDL) cholesterol (20). In terms of the current understanding of risk factors for coronary heart disease (CHD), increased LDL cholesterol and decreased HDL cholesterol are both believed to be unfavorable changes in blood lipoprotein profile (21). The findings of the 1990 study were corroborated in subsequent studies (22, 23), but there has been a lack of consensus regarding the significance of *trans* fatty acids in the diet in relation to the risk for development of CHD (7, 24, 25). A 1996 report of a task force organized by the American Society of Clinical Nutrition and the American Institute of Nutrition concluded that insufficient data from biological studies were available for dietary recommendations to be made (24). This report also emphasized the task force's concerns that undue emphasis on *trans* fatty acids might detract from efforts to reduce consumption of saturated fats, which also raise LDL cholesterol and are present at much higher levels in the diet. However, the controversy regarding the nutritional implications of *trans* fatty acids has led to speculation in recent years about the possibility of mandatory *trans* labeling (26-28), and this speculation, in turn, has fueled interest in the development of accurate and rapid analytical methods for the determination of *trans* content (29).

1.2. RATIONALE AND OBJECTIVES OF THE RESEARCH

IR spectroscopy has played a major role in fundamental research and qualitative analysis of lipid systems over the past five decades owing to the large amount of

structural information that can be extracted from the IR spectra of lipids. It has also been an important tool in the routine analysis of hydrogenated fats and oils because it has provided a relatively simple, although not highly accurate, means of determining the levels of *trans* isomers formed during catalytic hydrogenation processes. However, other quantitative analysis applications have been lacking, in large part because of the limitations of traditional dispersive IR spectroscopy. In 1992, the McGill IR Group initiated a research program whose aim was to expand the scope of quantitative IR analysis of fats and oils by fully exploiting the capabilities of FTIR instrumentation and the powerful chemometric techniques that had recently become available for multicomponent analysis and for the correlation of IR spectral data to physical properties and quality parameters. The focus of this research initiative was on the development of FTIR analytical methodology for routine quality control applications in the edible oil industry (6).

The research described in this thesis was an integral part of this research program and was undertaken shortly after its inception. Two major areas of potential application of FTIR spectroscopy in the routine analysis of fats and oils were selected for investigation: (i) the measurement of oxidative status and stability and (ii) the bulk characterization of fats and oils in terms of their degree and type (*cis* or *trans*) of unsaturation. The journal publications that resulted from this research are presented in Chapters 3-7 of this thesis, following a review of the relevant literature in Chapter 2.

The overall objective of the work described in Chapter 3 was to lay the foundation for the development of FTIR methods for assessing oil quality in relation to lipid oxidation and thermal stress. Although fundamental IR characterization and identification

of specific products formed from the oxidation of individual fatty acids and the decomposition of fatty acid hydroperoxides was achieved as early as the 1940s, no comprehensive IR spectroscopic investigations of edible oils undergoing oxidation had been reported in the literature. Thus, detailed FTIR studies were undertaken to characterize the spectral changes occurring as oil oxidation proceeds, assign wavelengths to the more common molecular species produced, and assess the potential utility of FTIR spectroscopy as a tool for monitoring oil oxidation and quantitating key products associated with oxidation.

The overall objective of the research described in the next four chapters was the development of FTIR methods for the determination of IV and *cis* content as well as of alternatives to the traditional IR method for the determination of *trans* isomers that overcome the well-established limitations of the latter method. The foundation for these studies was the work conducted by the McGill IR Group on the determination of IV and saponification number (SN) of fats and oils by FTIR/attenuated total reflectance spectroscopy (30). In Chapter 4, the development of a transmission FTIR method for the simultaneous determination of IV, *cis* and *trans* content, and SN based on a multivariate calibration approach is described. Chapter 5 concerns the modification of the traditional IR method for the determination of *trans* unsaturation to allow for direct analysis of oils and fats without derivatization or the use of a solvent. In Chapter 6, the results of a validation study involving the analysis of over 100 samples of partially hydrogenated oils by the methods discussed in Chapters 4 and 5 are presented. The research described in Chapter 7 stemmed from the adoption by the AOCS of a new FTIR method as a Recommended Practice for the determination of isolated *trans* isomers by IR

spectroscopy (31). This method, which was developed by researchers at the U.S. FDA (32) and became an AOCS Official Method in 1999, is based on the same principles as the method described in Chapter 5 of this thesis but specifies the use of a SB-HATR sample-handling accessory. In view of the potential widespread use of this method, particularly should the determination of *trans* content become a required analysis for compliance with nutrition labeling regulations, research was undertaken with the objective of developing an SB-HATR method for the simultaneous determination of IV and *trans* content. Following the description of this method in Chapter 7, overall conclusions and perspectives on the potential utility of FTIR spectroscopy as an analytical tool in the fats and oils industry are presented in the final chapter of the thesis.

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CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

The work described in this thesis addresses the possibility of replacing some of the methods traditionally employed in fats and oils analysis by FTIR-based methods. The first part of this chapter comprises a review of the methods employed in fats and oils analysis that are pertinent to the specific applications of FTIR spectroscopy considered in this thesis. The focus will be primarily on the official methods of the American Oil Chemists' Society (AOCS), with some limited coverage of alternative methods that have been described in the recent literature. First, methods for the assessment of oxidative status and stability will be surveyed in Section 2.2.1, which is intended to serve as background to the examination in Chapter 3 of the use of FTIR spectroscopy to monitor oil oxidation. This is followed in Section 2.2.2 by a review of methods for the determination of iodine value and *trans* content, as the research that will be described in Chapters 4-7 pertains to these particular analyses.

The second part of this chapter is devoted to IR analysis of edible fats and oils. An overview of the infrared spectral characteristics of lipids and a brief description of the principles of FTIR spectroscopy will be presented to serve as general background information. The quantitative analysis methods and sample-handling techniques that have been employed in the work described in this thesis will then be discussed. In the final section, the literature on the quantitative analysis of fats and oils by IR spectroscopy will be reviewed.

2.2. FATS AND OILS ANALYSIS

2.2.1. Methods to Assess Oxidative Status and Stability

For all unsaturated oils, the major cause of loss of quality subsequent to refining is lipid autoxidation, which gives rise to the off-flavors and unpalatable odors associated with oxidative rancidity. Thus, various analyses that indicate the extent of oxidation are commonly performed, sometimes in conjunction with accelerated oxidation tests for the purpose of predicting the shelf life of oils and fat-containing foods. In addition, lipid oxidation proceeds rapidly at frying temperatures, and monitoring of oil oxidation in industrial frying operations is critical in maintaining the quality of the product; the extent to which the oil in industrial fryers undergoes oxidation is also a major factor governing the shelf life of products prepared by deep-fat frying, such as fried snack foods (1). Measures of oxidative status and stability are also widely employed to evaluate the effectiveness of antioxidants in retarding lipid autoxidation.

Methods for the assessment of oxidative status and stability have been reviewed by Gray (2) and Robards *et al.* (1). Although a large variety of both chemical tests and instrumental methods have been described in the literature, a limited number of them are routinely employed in quality control. The AOCS's Recommended Practices for Assessing Oil Quality and Stability (3) comprise six methods, all of which are based primarily or exclusively on the measurement of oxidation products. Three additional AOCS official methods have also been extensively used for the assessment of oxidative status and stability. These nine methods are listed in Table 2.1 and will be briefly described in this section.

Table 2.1. AOCS Methods Employed for the Assessment of Oxidative Status and Stability[a]

AOCS method no.	Name of method	What is measured
Cg 2-83	*Flavor Panel Evaluation of Vegetable Oils*	Off-flavors and odors
Cd 8-53 & Cd 8b-90	*Peroxide Value*	Primary oxidation products
Ti 1a-64	Spectrophotometric Determination of Conjugated Dienoic Acid	Primary oxidation products formed from polyunsaturated fatty acids
Cd 18-90	*Anisidine Value*	Aldehydes (secondary oxidation products)
Cg 4-94	*Volatile Organic Compounds in Fats and Oils by Gas Chromatography*	Volatile secondary oxidation products and other volatiles
Cg 1-83	*Correlation of Oil Volatiles with Flavor Scores of Edible Oils*	Volatile secondary oxidation products responsible for off-flavors and odors
Cd 12-57	Fat Stability, Active Oxygen Method	Resistance to oxidation under accelerated oxidation conditions (97.8°C), as assessed by monitoring the formation of primary oxidation products
Cd 12b-92	*Oil Stability Index*	Length of induction period under accelerated oxidation conditions (100-140°C), as assessed by monitoring the formation of volatile organic acids (secondary oxidation products)
Cg 5-97	Oven Storage Test for Accelerated Aging of Oils	Resistance to oxidation under accelerated oxidation conditions (60°C), as assessed by sensory evaluation or by monitoring the formation of primary or secondary oxidation products

[a]Methods in italic type comprise AOCS Recommended Practice Cg 3-91: Recommended Practices for Assessing Oil Quality and Stability.

2.2.1.1. General Principles

All chemical methods for the assessment of the oxidative status and stability of oils are based on knowledge of the mechanism by which atmospheric oxygen reacts with lipids and the nature of the products formed. Because lipid autoxidation is a major deteriorative reaction affecting the quality of fats and oils, the mechanism has been the subject of extensive study, as reviewed by Frankel (4). The main oxidative pathway is via the formation of hydroperoxides, ROOH, through a free-radical mechanism (5):

Initiation: $RH \rightarrow R\bullet$

Propagation: $R\bullet + O_2 \rightarrow ROO\bullet$

$ROO\bullet + RH \rightarrow ROOH + R\bullet$

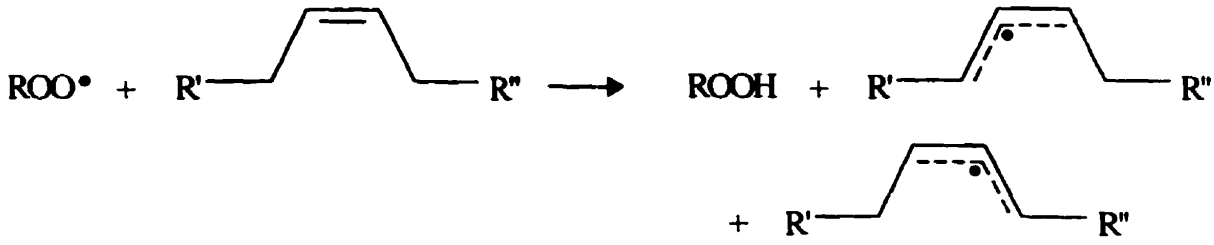
Termination: $R\bullet + R\bullet$

$R\bullet + ROO\bullet \rightarrow$ nonradical products

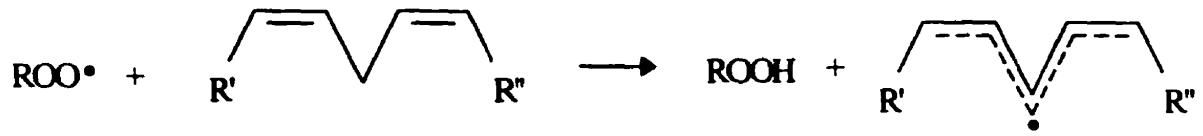
$ROO\bullet + ROO\bullet$

In the above reaction scheme, RH denotes an unsaturated lipid, such as the oleate, linoleate, and linolenate side chains that are commonly present in the triglycerides of vegetable oils. The initiation step, which involves loss of a hydrogen radical from an unsaturated lipid to form a lipid free radical, is catalyzed by a variety of agents (light, heat, metal ions, etc.). In the first of the propagation steps, the lipid free radical reacts with molecular oxygen to form a peroxyl radical. This reaction is much more rapid than the second of the propagation steps, the free-radical abstraction of hydrogen from the fatty acid chain of another unsaturated lipid molecule, forming a hydroperoxide and a new lipid radical. The hydrogen is abstracted from a carbon adjacent to a double bond

and the resulting allylic radical is stabilized by delocalization of electron density from the double bond:



Polyunsaturated fatty acids contain methylene-interrupted double bonds and are particularly susceptible to free-radical attack owing to the low bond dissociation energy of the allylic hydrogen at the carbon atom between the double bonds and the relative stability of the pentadienyl radical formed by abstraction of this hydrogen:



Thus, the relative rates of oxidation of methyl oleate, methyl linoleate, and methyl linolenate have been reported to be 1:41:96 (6), the relative rate for linolenate being approximately double that for linoleate because linolenate contains two pairs of methylene-interrupted double bonds.

The hydroperoxides formed by the above mechanism are unstable. Thus, these primary oxidation products break down to a variety of secondary oxidation products, including volatile products such as short-chain aldehydes, ketones, fatty acids, alcohols, and hydrocarbons (7). These volatile secondary oxidation products are responsible for the off-flavors and unpalatable odors associated with oxidative rancidity. In particular, many of the aldehydes formed have very low flavor thresholds; for example, in the oxidation of

soybean oil, the most significant flavor volatile has been identified as *trans*, *cis*-2,4-decadienal (8), which has a flavor threshold of 0.02 ppm (9). Because different distributions of secondary oxidation products, each having different flavor thresholds, are produced depending on the composition of the oil and the oxidation conditions, flavor stability differs from oxidative stability For instance, the flavor volatiles produced from oxidation of linolenate have lower threshold values than those produced from oxidation of linoleate, and hence polyunsaturated oils with high levels of linolenate, such as soybean and canola oil, will exhibit oxidative rancidity at an earlier stage of oxidation than those containing lower levels of linolenate (10).

2.2.1.2. Sensory Evaluation

In view of the distinction between oxidative and flavor stability discussed above, and the fact that the latter rather than the former governs the acceptability of oils for food uses, it is apparent that sensory evaluation is the optimum approach to the assessment of oil quality and stability in relation to autoxidation. A standardized technique for the evaluation of vegetable oils by a trained taste panel is provided by AOCS Recommended Practice Cg 2-83, which includes details of the preparation of reference standards, setup of the taste panel room, preparation and presentation of samples, and testing and scoring.

Although the inherent superiority of sensory evaluation as a means of assessing oil quality and flavor stability is evident, sensory analyses have a number of drawbacks, including cost, the need for proper facilities and properly trained taste panelists, and poor interlaboratory reproducibility (2). Accordingly, chemical and instrumental methods for

the assessment of oxidative status and stability are usually employed to evaluate oil quality and predict shelf life.

2.2.1.3. Peroxide Value

Although hydroperoxides are odorless and tasteless, the level of these primary oxidation products in an oil and its rate of change are important indicators of oil quality and potential shelf life (1). The standard method used in the edible oil industry to determine hydroperoxides is the AOCS iodometric method for the determination of peroxide value (PV) (11). The method is based on the stoichiometric conversion of KI to molecular iodine in an acidic medium and subsequent titration with sodium thiosulfate to determine the amount of molecular iodine released:

$$ROOH + 2H^+ + 2I^- \rightarrow I_2 + ROH + H_2O$$

$$I_2 + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I^-$$

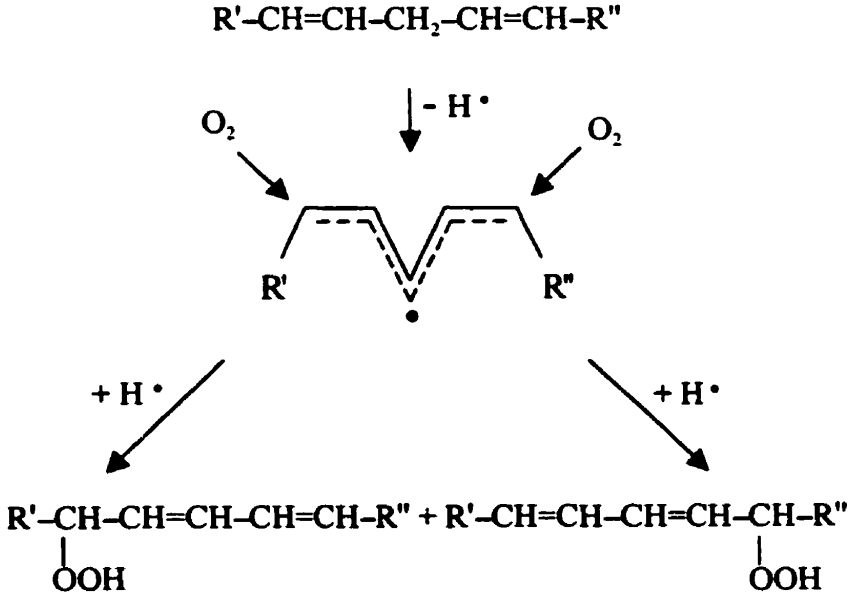
The PV is expressed as milliequivalents of iodine per kilogram of oil. In the original AOCS method (Cd 8-53), the above reaction is carried out in an acetic acid-chloroform solution. Owing to the toxicity and carcinogenicity of chloroform and the environmental concerns associated with its use, a second method (Cd 8b-90) was recently adopted in which chloroform is replaced by isooctane. This method is not recommended for the measurement of high-PV samples (PV $\geq$ 70), owing to erratic results attributed to the poor miscibility of isooctane and the aqueous titrant, resulting in a time lag in the liberation of iodine from the starch indicator (12).

PV is considered to be a useful measure of oxidative status in the initial stages of oxidation (13) and under mild conditions. However, when PV is monitored as a function

of oxidation time, it reaches a maximum and then decreases owing to the decomposition of hydroperoxides to secondary oxidation products (13). The thermal instability of hydroperoxides also results in their rapid decomposition at elevated temperatures. Thus, in the later stages of oxidation or under accelerated oxidation conditions (e.g., at temperatures above 100°C), the measured PV does not reflect the true extent of oxidation.

2.2.1.4. Conjugated Diene Value

As mentioned above, in polyunsaturated fatty acids, free-radical abstraction of the allylic hydrogen from the carbon atom between two double bonds to form a pentadienyl radical is highly favored. Insertion of molecular oxygen then takes place at either end of the pentadienyl radical, resulting in the formation of conjugated diene hydroperoxides:



The conjugated diene hydroperoxides are the predominant primary oxidation products formed during autoxidation of polyunsaturated fatty acids. For instance, when methyl linoleate was oxidized at temperatures in the range of 4-60°C, 9-OOH and 13-OOH

conjugated diene hydroperoxides accounted for approximately 98% of the monohydroperoxides formed (14). The conjugated diene hydroperoxides exhibit strong ultraviolet (UV) absorption at 234 nm, and the increase in the intensity of this absorption parallels the increase in PV during the initial stages of oxidation of polyunsaturated oils (15). Thus, measurement of the conjugated diene absorption by UV spectrophotometry provides a simple, reagent-free alternative to PV measurements for oils containing high levels of polyunsaturated fatty acids. However, because only polyunsaturated fatty acids give rise to conjugated hydroperoxides, the conjugated diene values obtained (expressed as a percentage of conjugated dienoic acid) are dependent on the initial fatty acid composition. Accordingly, these values do not serve as an unambiguous measure of the extent of oxidation and cannot be directly compared between oils of different types. Furthermore, beyond the initial stages of oxidation, secondary oxidation products such as conjugated dienals contribute to the UV absorption measured at 234 nm, and since the extinction coefficients of these compounds are different from those of the conjugated diene hydroperoxides, the conjugated diene value ceases to be a reliable indicator of the extent of oxidation. In addition, the method lacks specificity as conjugated dienes formed in small amounts during catalytic hydrogenation processes and conjugated trienes produced during high-temperature deodorization of linolenate-containing oils also absorb at 234 nm and thus contribute to the conjugated diene value.

2.2.1.5. Anisidine Value

As mentioned above, volatile aldehydes formed by the breakdown of hydroperoxides are major contributors to oxidative rancidity owing to their low flavor

thresholds. Thus, a number of chemical tests for the determination of aldehydes have been employed for the evaluation of the oxidative status and stability of oils. The most widely used of these is the anisidine value (AV) test, which is an official method of the AOCS (16). The AV test is based on UV detection of the products formed by the reaction between *p*-anisidine and aldehydes. All aldehydes do not contribute equally to the AV, as the molar absorptivity of the *p*-anisidine/aldehyde reaction products varies with aldehyde type. Thus, the AV test is particularly sensitive to conjugated dienals, which are major secondary oxidation products in oils containing linoleate and linolenate (8). The results of the AV test are often combined with those from PV determinations to yield a measure of oxidative status that takes into account both primary and secondary oxidation products, known as the Totox value [Totox = AV + (2 × PV)].

2.2.1.6. Gas Chromatography

Gas chromatography (GC) provides a means for direct measurement of the volatile compounds that are responsible for the off-flavors and odors associated with oxidative rancidity. AOCS Recommended Practice Cg 1-83, entitled "Correlation of Oil Volatiles with Flavor Scores of Edible Oils," outlines a procedure for utilizing GC data to assess oil quality and stability by generating correlation factors that relate total volatiles to taste panel results. Relationships between the levels of specific volatiles and the taste panel results may also be established by multiple linear regression, but the use of total volatiles is recommended for most applications. In either case, individual correlation studies, entailing GC analysis and sensory evaluation of samples stored under standardized conditions for various periods of time, are required for each particular oil

type and also for each processing treatment. For example, the correlation factors established for a hydrogenated soybean oil would not be applicable to a soybean oil hydrogenated under a different set of conditions.

As outlined in AOCS Recommended Practice Cg 4-94, three techniques may be employed in the GC analysis of the volatiles in oxidized oils: (a) direct injection; (b) static headspace; and (c) dynamic headspace. With direct injection, the oil sample is introduced into the gas chromatograph on a glass-wool plug placed in an injection port liner, thereby avoiding contamination of the column by the oil. At the elevated temperature of the injection port, volatiles are released from the oil onto the column. In the static headspace method, the sample is heated in a sealed container to drive the volatiles into the headspace until equilibrium between the oil and the gas phase is reached or approached. An aliquot of the headspace is then injected into the gas chromatograph. In the case of the dynamic headspace method, volatiles are driven into the gas phase by purging the heated oil sample with nitrogen or helium and are collected on a porous polymer trap. The volatiles are then desorbed from the polymer at elevated temperatures (150-230°C) and transferred into the gas chromatograph. In all three cases, the volatiles are concentrated at the head of the GC column by cryogenic cooling (-50°C), as this practice yields better separations.

GC analysis of an oxidized oil by each of the three techniques described above yields different flavor volatile profiles owing to the inherent differences between these techniques (8, 17). The static headspace technique favors the more volatile, lower-molecular-weight components because the equilibrium concentrations of volatiles in the

headspace above the sample depend on their vapor pressure as well their concentrations in the oil. Thus, the direct-injection and especially the dynamic headspace techniques have greater sensitivity in the analysis of less volatile components. In the dynamic headspace technique, there may be some loss of more volatile components owing to inefficient trapping on the polymer sorbent. The temperature at which the sample is heated is lower in the dynamic headspace technique than in the other two methods, thereby minimizing the decomposition of hydroperoxides and the resulting formation of additional flavor volatiles not originally present in the sample. For this reason, the dynamic headspace technique can provide a flavor volatile profile that better represents the compounds actually contributing to the flavor and odor of the oil at the time of testing (18). However, because of the longer time required for analysis with the dynamic headspace technique and the effort required to clean and maintain the equipment, this technique is generally not considered to be suitable for routine analyses.

2.2.1.7. Assessment of Oxidative Stability

The methods described above for the assessment of oil quality in relation to oxidative status are also often incorporated in tests of oxidative stability, which are performed to evaluate the resistance of an oil to autoxidation during storage and under the conditions to which it will be subjected in food manufacturing processes. Some of these tests are shelf-life tests, in which the oil is held under normal storage conditions and monitored at regular intervals for the development of rancidity, usually by sensory evaluation. However, since lipid autoxidation is characterized by a substantial induction period under such conditions, most oxidative stability tests involve conditions that

accelerate the oxidation process, such as elevated temperature, addition of metal catalysts, exposure to light, or oxygenation.

The most commonly employed method of evaluating oxidative stability has traditionally been the active oxygen method (AOM), which was an official method of the AOCS (19) prior to 1996, when it was declared obsolete. The protocol for the AOM involves maintaining the oil at 97.8°C while bubbling air through it at a specified flow rate. The change in the PV of the oil is monitored as a function of time, and the length of time required to reach a PV of 100 is taken as a measure of the oil's resistance to oxidation. This value is often included in the specifications of refined oils, particularly those sold for use as frying oils (20). Despite the widespread use of the AOM, the method is considered to have a number of drawbacks. These include the need to perform time-consuming PV determinations and the sensitivity of the method to variations in the temperature and air flow rate (21). In addition, the AOM may not provide information relevant to the oxidative stability of oils under normal conditions of storage owing to the elevated temperature employed (22).

The oil stability index (OSI) is an automated method similar in principle to the AOM that was recently adopted as an official method by the AOCS (23). The OSI utilizes instruments such as the Rancimat and the Oxidative Stability Instrument, in which the volatiles from a heated, aerated oil sample are passed through deionized water and the conductivity of the water is monitored as a function of time as the oil undergoes oxidation. A rapid rise in conductivity marks the end of the induction period. This increase in conductivity is due to the formation of short-chain organic acids, primarily

formic and acetic acid (24), as secondary oxidation products. The instruments used to determine the OSI automatically measure the inflection point of the conductivity versus time curve, which corresponds to the end of the induction period. In a report on a collaborative study of the OSI (21), a number of advantages of this method by comparison with the AOM were enumerated. Apart from the evident benefits associated with being an automated and reagent-free method, the OSI was claimed to be superior because it is based on the measurement of stable secondary oxidation products rather than the thermally unstable hydroperoxides measured in the AOM and, unlike the AOM, is not highly sensitive to variations in air flow rates. The results of the 15-laboratory collaborative study yielded a coefficient of variation of 11.3% for the OSI as compared to ~35% for the AOM (21). However, like the AOM, the OSI has been criticized because the course of lipid oxidation at the elevated temperatures required (97.8°C or higher) is different from that under normal conditions of oil storage (22).

Less severe conditions are employed to accelerate oxidation in the oven test (25). This test involves simply placing the sample in a forced-draft oven held at 60°C to assess the amount of time required for rancidity to develop. The importance of ensuring that the oven is free of contaminants, such as oxidation products of previously or concurrently analyzed samples, which may act as prooxidants, has been emphasized (26). The development of rancidity in the oven test may be assessed by sensory evaluation or by various chemical and instrumental methods for the evaluation of oxidative status (e.g., PV, AV, conjugated diene value, gas chromatography) (27). Because the oven test employs a moderate temperature, it is considered to be representative of normal storage

conditions, but consequently it also has the drawback of being fairly lengthy, as the time required for most oils to become rancid under the conditions of the test is on the order of 4-8 days (27).

The importance of selecting appropriate conditions for the evaluation of oxidative stability has been emphasized by several authors (28, 29). The conditions used to accelerate oxidation affect not only the rate of oxidation but also the rates and pathways of breakdown of the primary oxidation products, and hence alter the distributions of secondary oxidation products. Hence. accelerated oxidation tests conducted, for instance, at temperatures in excess of 80°C may not provide information relevant to the oxidative stability of the oil under conditions encountered during storage and food processing. As noted above, some of the most widely utilized oxidative stability tests, such as the AOM and the OSI, have been criticized in this regard. For similar reasons, the use of metal catalysts, enzymes, or irradiation to accelerate oxidation is generally not appropriate for the prediction of oil stability during storage and use (29). It has been suggested that the optimal means of accelerating oxidation for purposes of oxidative stability testing is by allowing for much greater access of oxygen to the oil than would be the case in bulk storage (29).

2.2.2. Measurement of Unsaturation

2.2.2.1. Iodine Value

One of the most widely performed analyses in the fats and oils industry is the determination of the degree of unsaturation, as measured by the iodine value (IV). The term iodine value derives from the reactivity of unsaturated compounds with molecular

iodine and other halogens, which add across carbon-carbon double bonds. The traditional methods for the determination of iodine value are all based on analytical iodometry, and the iodine value is defined as the number of grams of iodine that add to 100 g of sample. In the Wijs method, which is an official method of the AOCS (30), the sample is dissolved in carbon tetrachloride and reacted with an excess of iodine monochloride, which is added as a solution in glacial acetic acid (Wijs solution). After 30 min, a solution of potassium iodide is added to convert the unreacted reagent to molecular iodine, which is then titrated with standard sodium thiosulfate reagent using a starch indicator. This method yields accurate and reproducible results, provided that the experimental procedure is carefully followed (31). However, it has a number of disadvantages, including the relatively long total analysis time, the sensitivity of the Wijs solution to temperature, moisture, and light, and the hazardous nature of the Wijs solution and other noxious reagents employed in the analysis. Because of the toxicity and disposal problems associated with the use of chlorinated solvents, a revised procedure in which carbon tetrachloride is replaced by cyclohexane was recently adopted as AOCS Recommended Practice Cd 1b-87. The description of the method (32) indicates that longer analysis times may be required for high-IV samples (IV > 100) when cyclohexane is used as solvent.

Another AOCS official method, entitled "Calculated Iodine Value" (33), obtains IV from the fatty acid composition determined by GC. GC is a widely used and powerful technique for the qualitative and quantitative analysis of fatty acids in fats and oils, foods, and biological tissues. Its advantages include its specificity and sensitivity, with limits of quantitation on the order of 0.05%, and the small amounts of sample required (31). GC

analysis is usually not performed directly on triglycerides but on the more volatile fatty acid methyl esters (FAMEs), which are prepared either by acid-catalyzed transesterification of the triglycerides in the presence of excess methanol or by saponification of the triglycerides with alkali followed by esterification of the free fatty acids. In the official AOCS methods for the determination of fatty acid composition by GC, the sample is saponified by refluxing in 0.5N methanolic NaOH solution for 5-10 min; the free fatty acids are then esterified with BF_3-methanol reagent. AOCS Official Method Cd 1c-85 indicates that any of the several AOCS GC methods, using either packed or capillary columns, may be employed to obtain the fatty acid compositional data required for the calculation of IV in accordance with the following equation: IV = (% hexadecenoic acid × 0.950) + (% octadecenoic acid × 0.860) + (% octadecadienoic acid × 1.732) + (% octadecatrienoic acid × 2.616) + (% eicosenoic acid × 0.785) + (% docosenoic acid × 0.950). Although the determination of IV by GC is time-consuming and labor-intensive, it appears to be a widespread practice in QC laboratories.

As discussed above, the measurement of changes in unsaturation is important in monitoring catalytic hydrogenation processes. The time and effort required to perform GC analyses make the determination of IV by GC impractical for this purpose. The iodometric IV method described above is not suitable either, owing to the 30-min reaction time required, but the reaction time can be reduced to 3 min with the use of mercuric acetate as a catalyst. However, the difficulty of disposing of mercury wastes is an obvious drawback of this procedure. Accordingly, a more common practice in the industry is to employ refractive index measurements using an Abbé refractometer (34) as

a rapid means of monitoring changes in unsaturation during a hydrogenation process (35). Refractive index decreases as the degree of unsaturation decreases, and, under a fixed set of conditions, a good correlation exists between the refractive index and the iodine value (36). The Abbé refractometer cannot be employed for on-line analysis because light scattering by the catalyst particles in the oil interferes with the refractive index measurement. Recently, however, Cole *et al.* (37) reported an automated method based on the use of an optical waveguide which makes it possible to make on-line refractive index measurements without halting the hydrogenation process or filtering the oil to remove catalyst particles. A commercial on-line fiber-optic refractometer has also been described in a review article (35).

Although refractive index measurements can be employed to track changes in IV, no generally applicable relationship between these two parameters exists because the refractive index depends on a number of factors other than the degree of unsaturation, including the weight-average molecular weight of the oil and the free fatty acid content (36). Thus, refractive index measurements cannot serve as a substitute for IV determinations in QC analysis of fats and oils.

Several other instrumental methods have been investigated recently as possible alternatives to wet chemical methods for the determination of IV. Haryati *et al.* (38) proposed the use of differential scanning calorimetry (DSC) to measure the IV of palm oil. They showed that the DSC thermograms of palm oil samples showed a clear separation between peaks due to high- and low-melting triglycerides and that the relative sizes of these peaks were related to IV. Using stepwise regression analysis, they obtained an equation relating IV to four variables, corresponding to heights or areas of the peaks in

the DSC heating and cooling thermograms. On the basis of the results obtained, the authors concluded that DSC can be used to determine the IV of palm oil. Miyake *et al.* (39) investigated the possibility of determining IV by ^1H nuclear magnetic resonance (NMR) spectroscopy. In this work, the number of double bonds in the sample was determined from the areas of the signals due to olefinic or allylic plus divinyl protons, and the result was combined with the weight-average molecular weight of the sample, also determined from the NMR spectrum, to calculate IV. Linear regression of the calculated IVs for a set of vegetable oils against their chemical IV values yielded the following correction equation:

$$\text{Adjusted IV} = 0.9885 \times \text{Calculated IV}_{\text{NMR}} + 2.8084$$

The values obtained from the above equation were within ±1 unit of the chemically determined values. The measurement time of the NMR method was 3 min/sample.

Both IR spectroscopy and the complementary technique of Raman spectroscopy have also been suggested as techniques for the determination of IV. Work in this area will be presented below (Section 2.3.5.2) in the discussion of IR analysis of edible fats and oils.

2.2.2.2. trans Content

The official AOCS methods for the determination of the *trans* content of fats and oils are based on gas chromatography and IR spectroscopy. Until recently, the *trans* values provided by GC and IR analysis were not in good agreement, and neither technique was considered to measure *trans* content accurately (40). Modified versions of both the GC and the IR method have recently been adopted by the AOCS, and

preliminary findings indicate that these modifications have improved the concurrence between GC and IR *trans* data (40). As the IR methods will be described in detail in Section 2.3.5.1, only the GC methods will be considered here.

As mentioned above in relation to the GC determination of IV, GC is a widely employed technique for the determination of fatty acid composition. With the use of capillary columns coated with highly polar liquid phases, fatty acids can be readily separated according to chain length and degree of unsaturation. However, difficulties are encountered in resolving geometric and positional isomers (41, 42), and problems of peak overlap are particularly severe in the analysis of partially hydrogenated oils owing to the large number of positional and geometric isomers that are generated in hydrogenation processes. AOCS Official Method Ce 1c-89, which was until recently the only AOCS method for the determination of *trans* isomers by capillary-column GC, is based on separation of fatty acid methyl esters (FAMEs) on a 60 m × 0.25 mm i.d. fused-silica capillary column coated with SP-2340 (a cyanoalkylpolysiloxane liquid phase). Ratnayake and Beare-Jones conducted a detailed examination of the separation of C_{18} *cis*- and *trans*-unsaturated FAMEs on this type of column (43). They reported that excellent separation was obtained between *cis* and *trans* monounsaturated FAMEs having the double bond in the same position [e.g., methyl elaidate (18:1Δ9*t*) and methyl oleate (18:1Δ9*c*)], with the *trans* isomer always eluting before the *cis* isomer, but that peak overlaps occurred between some pairs of *cis* and *trans* isomers having double bonds at different positions. Among *cis* or *trans* positional isomers, those with the double bond nearer the methyl end of the fatty acid chain had longer retention times, such that a *trans*

isomer having a double bond at position Δy coeluted with a *cis* isomer having a double bond at position Δy – 6; for example, 18:1Δ12*t* coeluted with 18:1Δ6*c* and 18:1Δ15*t* coeluted with 18:1Δ9*c*. Similar problems with overlap were found for the 18:2 isomers; for example, 18:2Δ6*c*, Δ9*t*, 18:2Δ6*c*, Δ12*t*, 18:2Δ9*t*, Δ12*t*, and 18:2Δ9*t*, Δ15*t* could not be resolved, nor could 18:2Δ9*c*, Δ12*t*, 18:2Δ9*c*, Δ15*t*, and 18:2Δ12*t*, Δ15*t*. In terms of the accuracy of the GC method, the most significant effect of these *cis*/*trans* overlaps is that the peaks due to some of the 18:1*t* isomers will be under the peak due to 18:1Δ9*c*, which is the predominant 18:1*c* isomer in partially hydrogenated vegetable oils (44). As a result, the GC analysis underestimates *trans* content, with the magnitude of the error depending on the amounts of the various *trans* positional isomers present in the sample, which in turn depend on both the hydrogenation conditions and the composition of the source oil. For some samples, the error can be appreciable: the underestimation of 18:1*t* in margarine samples analyzed by AOCS Official Method Ce 1c-89 was reported to be as high as 32% (44).

Recently, Duchateau *et al.* reported that they had achieved adequate separation of *cis* and *trans* isomers by careful optimization of the GC conditions (45). With the use of a simulation program, they identified the optimum temperature for the separation of *cis* and *trans* isomers on three highly polar stationary phases under isothermal conditions. Experimental fine-tuning of the optimum temperature resulted in only very slight adjustments of the values obtained from the simulations, and the authors concluded that the corrections required to compensate for batch differences between columns would be on the order of 1°C. Their work is the basis for AOCS Official Method Ce 1f-96, which

was recently adopted for the determination of *cis* and *trans* fatty acids in hydrogenated and refined oils and fats (46). The method lists the optimized GC conditions (optimum isothermal temperature conditions, column head pressure, and carrier gas velocity) for the separation of *cis* and *trans* isomers on the three highly polar stationary phases investigated by Duchateau *et al.* (45) and describes the procedure for their identification and quantitation as well as for checking the performance of the GC system. For the analysis of partially hydrogenated oils, the performance check is based on a visible separation on the chromatogram of the peaks due to 18:1Δ9*c* and 18:1Δ13*t*. Such a separation is a prerequisite for an accurate split between *cis* and *trans* peaks.

A new AOCS Recommended Practice for *trans* analysis (47) utilizes silver-ion exchange high-performance liquid chromatography (HPLC). This method is based on the well-established utility of silver-ion chromatography for the separation of *cis* and *trans* isomers. Silver ions, like other transition-metal ions, form charge-transfer type complexes with unsaturated compounds, via the donation of electrons from the filled π orbitals of the double bond to the vacant 5*s* and 5*p* orbitals of the silver ion and the back-donation of electrons from the filled 4*d* orbitals of the silver ion to the vacant antibonding π* orbitals of the double bond (48). Because the complexes formed are unstable, the interaction of silver ions with double bonds can be exploited in chromatography to separate compounds according to their degree of unsaturation. Moreover, the stability of the complexes is greater for *cis* double bonds than for *trans* double bonds (49), and this is the basis for the separation of *cis* and *trans* isomers by silver-ion chromatography.

The above principles have been applied in both thin-layer (TLC) and column chromatography for several decades and, more recently, in HPLC. In most procedures, the stationary phase is impregnated with a silver salt, most commonly silver nitrate, although reversed-phase separations in which a silver salt is added to the eluent have also been performed (49). In the IUPAC method for the determination of *trans*-octadecenoic acids (50), fats and oils are converted to their methyl esters, which are then separated by TLC on silica impregnated with silver nitrate. The band corresponding to the *trans*-octadecenoates is collected and quantitatively extracted into diethyl ether in the presence of methyl heptadecanoate, added as an internal reference standard. The extracted mixture is then analyzed by GC. Although this procedure is fairly straightforward, it is too time-consuming to be suitable for routine analyses, and this is presumably the reason why it has not been adopted as an official method by the AOCS or the AOAC.

The use of HPLC in place of TLC provides a means of accelerating this method. For example, Toschi *et al.* (51) successfully separated the methyl esters of *trans*- and *cis*-monoenoic fatty acids on short (5 cm) silver-ion columns with dichloromethane/1,2-dichloroethane (1:1) as the mobile phase; with the addition of acetonitrile (0.005-0.01 mL/liter) to the mobile phase as a polar modifier, retention times were greatly reduced, allowing separations to be achieved in a few minutes. Two fractions were collected and subjected to GC analysis; the first fraction comprised the saturated and *trans*-monoenoic FAMEs, and the second the *cis*-monoenoic FAMEs. Dienoic and polyenoic FAMEs were not eluted under the conditions employed. The ratio of *trans* to saturated FAMEs in the first fraction was determined by GC analysis, and then the absolute amount of *trans* FAMEs was calculated by multiplying this ratio by the amount of saturated FAMEs, as

determined by GC analysis of the unfractionated sample. Although this method has the advantage of being more rapid than the combined TLC/GC method, it is still a fairly complicated procedure that can only be performed by a highly skilled analyst, which limits its utility for routine analyses.

2.3. INFRARED ANALYSIS OF EDIBLE FATS AND OILS

2.3.1. Infrared Spectral Characteristics of Lipids

The investigation of the infrared spectral characteristics of lipids dates back to the beginning of this century, when the pioneering studies demonstrating the potential general utility of IR spectroscopy as a tool for qualitative analysis were performed. In fact, the first compilation of IR spectra, published by Coblentz in 1905 (52), included the spectra of several fatty acids and vegetable oils, and a paper entitled "The Infra-red Spectra of Vegetable Oils" also appeared in the early literature on IR spectroscopy (53). However, major progress in the interpretation of the IR spectra of lipids was not made until the late 1940s and early 1950s, a period that represents the beginning of modern IR spectroscopy, as its extensive use during World War II by chemists engaged in war-related research efforts led to the commercial availability of IR spectrometers and their widespread utilization in the organic research laboratory.

In this postwar period, a number of researchers conducted detailed studies of the IR spectra of pure reference materials in order to build the necessary knowledge base for the application of IR spectroscopy in fats and oils analysis. For example, Shreve *et al.* (54) recorded the spectra of a number of pure fatty acids, methyl esters, triglycerides, and alcohols; liquids were scanned neat, and solids were dissolved in carbon disulfide. Band

assignments were made by comparison of the spectra of these various samples and through the use of published frequency correlation charts. An important result of this work was the identification of an absorption band at 10.36 μm (965 cm^{-1}) as characteristic of *trans* monounsaturation, which led these authors to develop an IR method for the determination of *trans* content (55). An analytical procedure based on the principles developed in this early work is still widely used by the fats and oils industry for the determination of *trans* isomers, particularly for the characterization of partially hydrogenated oils.

In recent years, several detailed examinations of the FTIR spectra of a variety of oils and fats, recorded in either the transmission mode (56, 57) or by the attenuated total reflectance technique (58), have appeared in the literature. Figure 2.1 shows the FTIR spectrum of a partially hydrogenated soybean oil in a 0.025-mm transmission cell; as the bands in the 3000-2850 cm^{-1} region are off-scale in this spectrum, the inset shows this spectral region recorded with a pathlength of 0.010 mm. The corresponding band assignments, which were established in the fundamental studies of the IR spectra of fatty acids and esters and triglycerides conducted in the 1950s, are tabulated in Table 2.2. Most of the bands in the so-called fingerprint region (1500-900 cm^{-1}) are not assigned in this table because more than one normal vibrational mode generally contributes to absorptions in this region of the spectrum. Small variations in the band positions listed in Table 2.2 may occur depending on the triglyceride composition of the oil (56). For example, for pure triglycerides, the position of the band due to the CH stretching vibration of *cis* double bonds depends on the number of double bonds in the fatty acid chain, shifting

from 3005 cm^{-1} for triolein (18:1*c*) to 3009 cm^{-1} for trilinolein (18:2*c*) and 3011 cm^{-1} for trilinolenin (18:3*c*).

More complicated spectra are obtained in the solid state, and these spectra can yield additional valuable information. In early work, Sinclair *et al.* conducted a detailed examination of the IR spectra of saturated fatty acids and esters (59). They found no qualitative differences between the spectra of fatty acids of different chain lengths in solution; however, the spectra recorded from samples in the crystalline state showed major differences with changes in chain length. In particular, a progression of absorption bands of uniform spacing (~20 cm^{-1}) was observed between 1350 and 1180 cm^{-1}, with the number of bands in the progression increasing as a function of chain length (60). These band progressions, and similar band progressions observed between 1070 and 710 cm^{-1}, are assigned to wagging and twisting-rocking vibrations of methylene groups and arise from different phase relationships between vibrations of adjacent methylene groups in the fatty acid chains. They are not seen in pure liquid or solution spectra because of the free rotation about the C–C bonds and the random motion of the fatty acid chains. In subsequent work on the salts of fatty acids, Kirby *et al.* (61) demonstrated that in the case of monounsaturated fatty acids the band progressions could be utilized to identify the position of the double band in the chain. Thus, these band progressions proved highly useful in the elucidation of the structure of unknown fatty acids isolated from various plant sources.

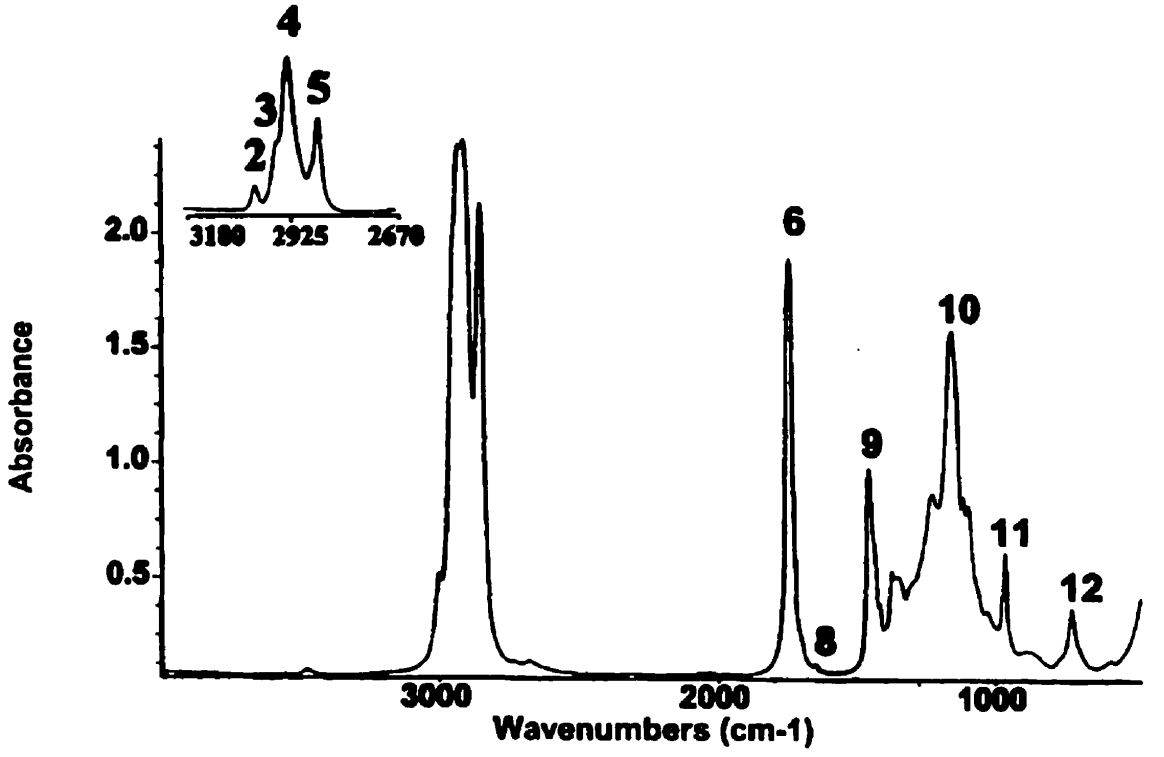


Figure 2.1. FTIR spectrum of a partially hydrogenated soybean oil in a 0.025-mm transmission cell. The inset shows the C–H region of the spectrum recorded in a 0.010-mm transmission cell. The C–H and C=C stretching absorptions of *trans* double bonds (bands 1 and 7, respectively, in Table 2.2) are not visually discernible in the spectrum of this sample (*trans* content of the sample is ~40%).

Table 2.2. Characteristic IR Absorption Bands of Vegetable Oils

Band[a]	Band position (cm^{-1})	Assignment
1	3025	ν_{sym}(=C–H) (*trans* double bonds)
2	3008	ν_{sym}(=C–H) (*cis* double bonds)
3	2953	ν_{asym}(C–H) (CH_3 groups)
4	2925	ν_{asym}(C–H) (CH_2 groups)
5	2854	ν_{sym}(C–H) (CH_2 and CH_3 groups)
6	1746	ν (C=O) (ester linkage)
7	1666	ν (C=C) (*trans*)
8	1654	ν (C=C) (*cis*)
9	1459	CH_2 scissoring deformation
10	1161	ν (C–O)
11	966	Out-of-plane =C–H deformation (*trans*)
12	722	CH_2 rocking

[a]Numbers refer to bands labeled on Figure 2.1, except for bands 1 and 7, which are not sufficiently intense to be discerned in the figure.

Solid-state spectra can also be used to differentiate the polymorphic forms in which fats crystallize (62). In particular, the splitting of the band at 720 cm^{-1} due to the CH_2 rocking mode into a doublet distinguishes the β′-form from the α- and β-forms. This splitting is associated with the orthorhombic packing of the β′-form and is due to interaction between neighboring chains. Thus, IR spectroscopy can be employed to investigate the polymorphic transformations of fats (63), which is important from a practical perspective since the crystal form of a fat affects its melting behavior and texture, the β′-form being the desired form for the production of margarines and shortenings because its small, fine crystals impart a smooth and creamy texture.

Because of the large amount of qualitative information that can be extracted from the IR spectra of lipids, IR spectroscopy has historically played a major role in fundamental research on the chemistry of fats and oils (64, 65). For example, based on the foundation laid by the work of Feuge *et al.* (66), who measured *trans* isomer formation during hydrogenation of methyl linolenate under various experimental conditions, IR spectroscopy was employed extensively in the study of *trans* isomer formation in catalytic hydrogenation processes as a function of various parameters, such as temperature, nature of the catalyst and the solvent, composition of the oil, and dispersion of hydrogen gas in the oil. IR spectroscopy also contributed substantially to the detailed understanding of the mechanisms of lipid autoxidation, by providing information on the structures of the hydroperoxides formed as primary oxidation products as well as on the identity of secondary oxidation products formed by the breakdown of hydroperoxides (67). In contrast, quantitative analysis applications, which will be the

focus of the work reviewed in this section, have been much more limited, with most of these applications having been developed since the advent of FTIR spectroscopy.

2.3.2. Instrumentation

An IR spectrometer essentially consists of a source of continuous infrared radiation, a means for resolving the infrared radiation into its component wavelengths, and a detector. The first commercial scanning IR spectrometers, which became available in the 1940s, employed a prism to resolve the infrared radiation into its component wavelengths. In instruments manufactured in the late 1950s and 1960s, the prism was replaced by a diffraction grating, leading to improved resolution. A more drastic change in instrument design occurred in the late 1960s when the principles of interferometry and Fourier transform spectroscopy were applied to the design of IR spectrometers. The development of these FTIR spectrometers, and the subsequent decrease in their cost, has almost eliminated the use of dispersive instruments and has increased the utility of IR spectroscopy as an analytical tool (68).

An interferometer is the central component of an FTIR spectrometer. Interferometry provides a means of encoding both frequency and intensity information in the signal that reaches the detector, thereby eliminating the need for a dispersive element, and the Fourier transform provides the means of decoding this information. The Michelson interferometer, which is the type of interferometer employed in most FTIR spectrometers, uses a beamsplitter to divide the beam of radiation from the IR source into two parts, one part being reflected to a stationary mirror and the other part being transmitted to a moving mirror (Figure 2.2). When the beams are reflected back, they

recombine at the beamsplitter, producing a constructive/destructive interference pattern due to the varying difference between the distances traveled by the two components of the beam, and part of the recombined beam then passes to the detector. After the infrared energy has been selectively absorbed by a sample placed between the beamsplitter and the detector, fluctuations in the intensity of the energy reaching the detector are digitized in real time, yielding an *interferogram* (Figure 2.3). The interferogram contains all the information that is required to produce the IR spectrum of the sample, but this information is in the time domain. In order to obtain a conventional IR spectrum, the interferogram is converted to the frequency domain by Fourier transformation. In FTIR spectroscopy, the interferogram is usually a plot of intensity as a function of the path difference between the stationary and the moving mirror, known as the retardation δ, which is proportional to time t because the moving mirror travels at constant velocity v:

$$\delta = 2vt \text{ centimeters} \qquad [2.1]$$

Fourier transformation of the interferogram $I(\delta)$ then yields a spectrum with the x axis in units of wavenumbers (cm^{-1}), $I(\bar{\nu})$, in accordance with the following relationship (69):

$$I(\delta) = 0.5H(\bar{\nu})I(\bar{\nu}) \cos 2\pi\bar{\nu}\delta \qquad [2.2]$$

where $H(\bar{\nu})$ is a single wavenumber-dependent correction factor that accounts for instrumental characteristics. Thus, it was only with the development of the Cooley-Tukey fast Fourier transform (FFT) algorithm and, beginning in the 1970s, with the widespread availability of minicomputers in the laboratory that FTIR spectroscopy became practicable.

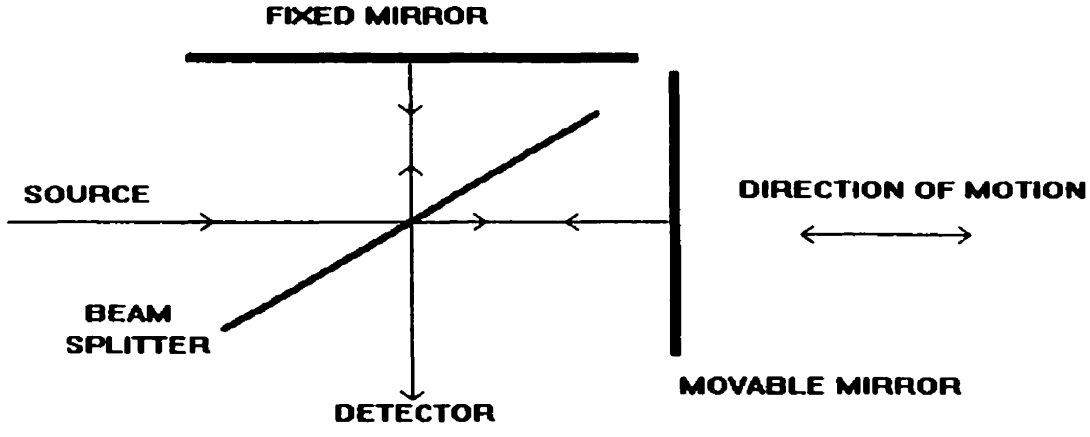


Figure 2.2. Schematic diagram of a Michelson interferometer.

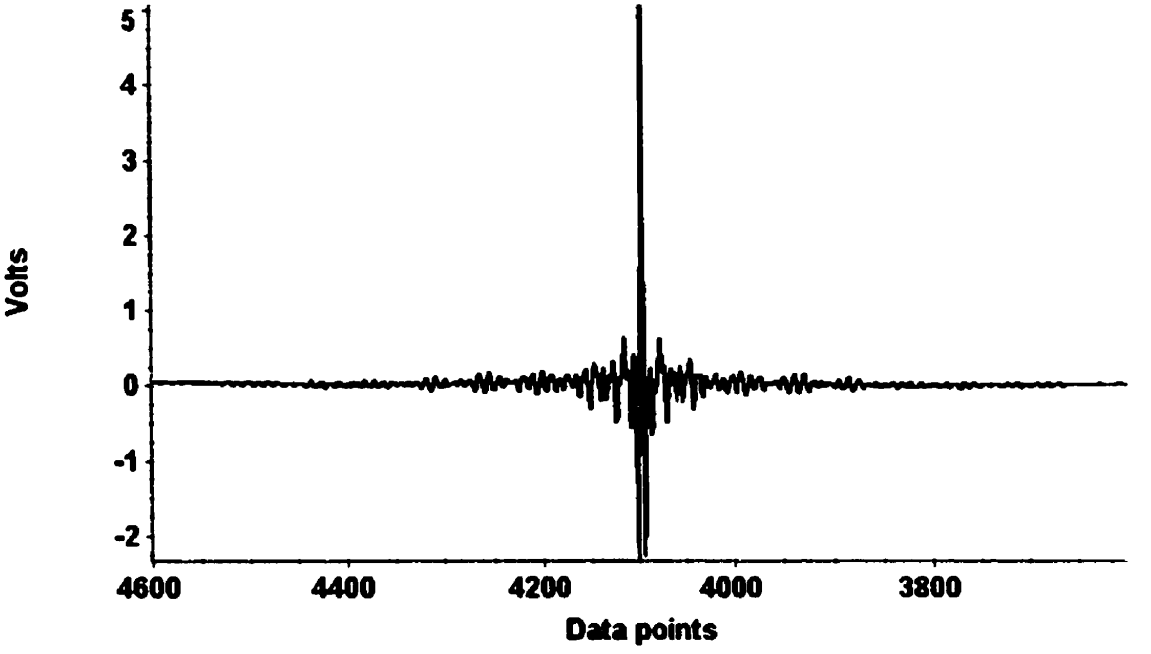


Figure 2.3. An interferogram recorded by an FTIR spectrometer.

The major reasons for the present dominance of FTIR instrumentation are the significant advantages that interferometers have over dispersive instruments (69). In an interferometer, all frequencies are measured simultaneously (the multiplexing or Fellgett advantage), and thus the entire FTIR spectrum of a sample can be collected in a single one-second scan. In addition, because the signal-to-noise ratio (S/N) of a spectrum increases as the square root of the number of scans, the multiplexing advantage also allows high S/N to be achieved in much shorter times than required with dispersive spectrometers. FTIR spectrometers also have higher energy throughput (the Jacquinot advantage) and are therefore more versatile in terms of the types of samples and sample-handling techniques that can be employed. Another important advantage for data manipulation and quantitative applications is that wavelength precision is maintained by using an internal reference laser (the Connes advantage), and thus wavelength drifts over time are eliminated as a possible source of error. The substantial computing power of FTIR systems has also been an important factor in the success of FTIR spectroscopy. FTIR software packages provide a wide variety of data handling routines that facilitate spectral acquisition and interpretation as well as powerful chemometric tools that have substantially enhanced the utility of IR spectroscopy as a quantitative analysis tool.

2.3.3. Quantitative Analysis

2.3.3.1. General Principles

As IR spectroscopy is a secondary method of analysis, the development of quantitative analysis methods requires calibration with a set of standards of known composition, prepared gravimetrically or analyzed by a primary chemical method, in

order to establish the relationship between IR band intensities and the compositional variable(s) of interest. Various calibration approaches may be employed, ranging from a simple Beer's law plot, which is an adequate basis for calibration in the case of a simple system, such as a single component dissolved in a noninteracting solvent, to the sophisticated multivariate analysis techniques that are required for more complex systems. The data handling capabilities of FTIR systems have allowed these latter techniques to be implemented in the instrument software and applied directly to spectral data, bringing a resurgence to quantitative IR spectroscopy during the past decade (70).

As with other types of absorption spectroscopy, the basis of quantitative analysis in IR spectroscopy is the Bouguer-Beer-Lambert law or Beer's law:

$$A_{\bar{\nu}} = \varepsilon_{\bar{\nu}} bc \qquad [2.3]$$

Here, $A_{\bar{\nu}}$ is the absorbance measured at wavenumber $\bar{\nu}$, $\varepsilon_{\bar{\nu}}$ is the molar absorption coefficient of the absorbing species at this wavenumber, b is the pathlength of the IR cell, and c is the concentration of the absorbing species. Application of Beer's law for the determination of the amount of a compound present in a sample requires that $\varepsilon_{\bar{\nu}}$ be determined by measuring the absorbance of a calibration standard of known concentration. Of course, in order to attain better accuracy, it is preferable to prepare a series of standards of different concentrations, spanning the concentration range of interest, and obtain $\varepsilon_{\bar{\nu}} b$ from a plot of absorbance versus concentration by linear least-squares regression. This procedure averages out the errors due to instrumental noise, measurement errors, and other sources of random variation. In addition, it allows deviations from Beer's law in the concentration range of interest to be detected, such as

may arise from hydrogen bonding, dimerization, and other intermolecular interactions. Such interactions may then be modeled by the introduction of higher order terms into the equation relating absorbance to concentration.

When the concentration of more than one species will vary in the samples to be analyzed, a multivariate calibration approach may be required, because the above univariate approach cannot account for any contributions of additional components to $A_{\bar{\nu}}$, nor can it model interactions between components. A variety of multivariate calibration techniques have been applied in the analysis of multicomponent systems, including the **K**-matrix (71) and **P**-matrix (72) methods and principal component regression (PCR) (73) and partial-least-squares (PLS) regression (73-75), both of which are forms of factor analysis. Among these various approaches, PLS has emerged as the technique of choice in the FTIR analysis of complex multicomponent systems. It has been applied, for instance, in the determination of the concentrations of three additives in polyethylene (76), the analysis of thin-film dielectrics (77), the determination of six components in detergents (78), the monitoring of incinerator emissions (79), the determination of fat, protein, lactose, and total solids in milk (80), and the determination of total protein, glucose, total cholesterol, triglycerides, and urea in human blood plasma (81). PLS is now included in most, if not all, commercial software packages for IR quantitative analysis.

2.3.3.2. Partial-Least-Squares (PLS) Regression

A PLS calibration model is developed by compressing the spectral data for the calibration standards into a set of orthonormal basis vectors, known as the loading spectra or factors. The mathematical procedure by which basis vectors are selected to model the

spectra of the calibration standards emphasizes the spectral variations due to differences in the concentration of the component of interest; this represents the fundamental difference between PLS and PCR. The spectrum of each calibration standard is then decomposed into a weighted sum of the loading spectra, and the weights given to each loading spectrum, known as "scores", are regressed against the concentration data for the standards. Thus, for m standards, l frequencies, and r factors:

$$\mathbf{A} = \mathbf{BT} + \mathbf{E}_A$$

$$\mathbf{c} = \mathbf{vT} + \mathbf{e}_c$$

where $\mathbf{A}$ is the $l \times m$ matrix of absorbance values, $\mathbf{B}$ is the $l \times r$ matrix of loading spectra, $\mathbf{T}$ is the $r \times m$ matrix of scores, $\mathbf{E}_A$ is the $l \times m$ matrix of errors in absorbance, $\mathbf{c}$ is the vector (of length m) of concentrations of the component of interest in the standards, $\mathbf{v}$ is the vector (of length r) of regression coefficients relating the scores to the concentrations, and $\mathbf{e}_c$ is the vector (of length m) of concentration errors (74). In the prediction step, the amounts of each loading spectrum employed in reconstructing the spectrum of the unknown, i.e., the "scores", are used to predict the concentration of the unknown. The above equations are those for PLS1, in which each component is considered independently. Using PLS2, a calibration model for multiple components can be developed; however, PLS1 has been reported to have better predictive properties (82).

Overfitting of the spectral data is always possible in developing a PLS calibration model, as the fit between the actual and the predicted values for the calibration standards will necessarily be improved as the number of loading spectra included in the model is increased, a perfect fit being achieved when the number of loading spectra equals the

number of calibration standards. However, because the concentration data for the standards are used in generating the loading spectra, most of the spectral variability associated with the concentration variations is incorporated in the early loading spectra (74). Thus, the later loading spectra will mostly represent noise in the spectra of the calibration standards or spectral information that is unrelated to concentration, and their inclusion in the PLS calibration model will deteriorate its performance in the prediction of unknowns and will also tend to make the model less robust (74, 83). Thus, validation of a PLS calibration model is always required in order to select the optimum number of loading spectra. This may be achieved by testing the performance of the model with standards not included in the calibration set, but this approach has the disadvantage that the development of the model becomes tailored to the test set. Alternatively, the "leave-one-out" cross-validation technique may be employed, whereby the calibration is performed m times with m - 1 standards and the mth standard is predicted as an unknown. The predicted residual error sum of squares (PRESS) is then computed from the errors in the predictions obtained for the m standards by cross-validation and plotted as a function of the number of factors included in the calibration model (Figure 2.4). Various criteria for selecting the optimum number of factors by examination of the PRESS plot have been discussed by Haaland and Thomas (74). These authors suggested that the option of selecting the number of factors corresponding to the minimum of the PRESS plot could lead to overfitting because the PRESS is based on a finite number of samples and is therefore subject to error. They recommended that an F-test be applied to evaluate the statistical significance of decreases in PRESS with increasing number of factors. The

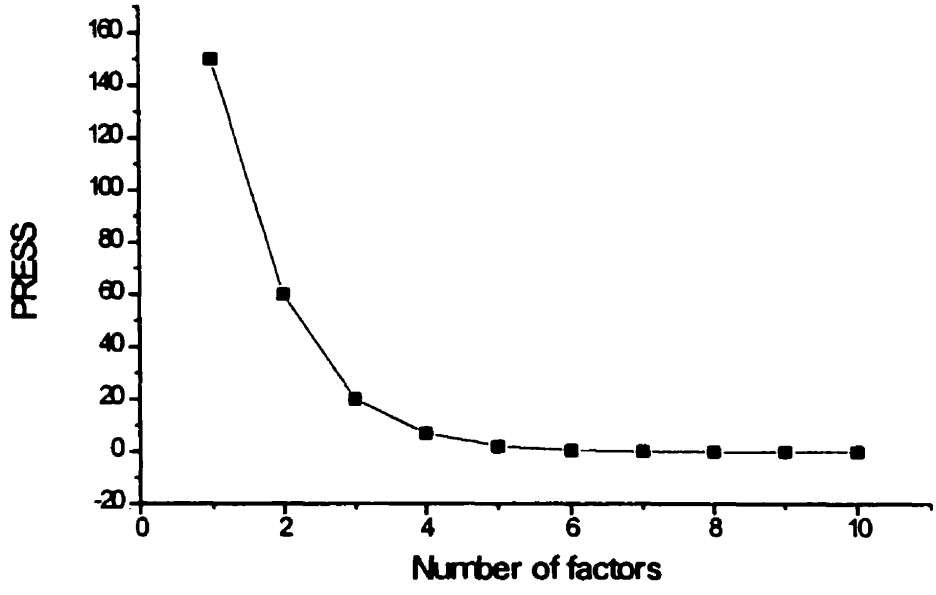


Figure 2.4. Typical PRESS plot obtained from cross-validation of a PLS calibration, showing the predicted residual error sum of squares (PRESS) as a function of the number of factors included in the calibration model.

optimal number of factors would then be the smallest number for which the PRESS is not statistically greater than that at the minimum of the PRESS plot. Several other approaches to the selection of the optimal number of factors have also been described in the literature (83).

A PLS calibration can, in principle, be based on the whole spectrum, although in practice the analysis is restricted to regions of the spectrum that exhibit variations with changes in the concentration of the component of interest. As such, the use of PLS can provide significant improvements in precision relative to methods that use only a limited number of frequencies (82). Furthermore, PLS is able to compensate for spectral interferences such as overlapping bands and band shifts due to intermolecular interactions, provided all sources of such interferences that may be present in the samples to be analyzed are present in the calibration standards. The powerful data reduction capabilities of PLS can also be exploited to establish relationships between physicochemical properties or quality attributes and FTIR spectral data (74). In this context, the term "quality attribute" refers to a parameter that is not a direct measure of the concentration of a particular component in the sample but is related in some manner to the concentrations of several components or to the overall composition of the sample. Although similar correlations can be achieved through the use of multiple linear regression, by establishing relationships between the value of a quality attribute and absorbance values at several frequencies, such an approach requires that the compositional variables contributing to the quality attribute be identified. By contrast, PLS can be used to develop a calibration model for the prediction of a quality attribute without any prior knowledge of the underlying relationship between its value and compositional variables.

2.3.4. Sample Handling

Quantitative IR analysis of fats and oils has traditionally involved dissolution of the sample in a suitable solvent, such as carbon disulfide or carbon tetrachloride, for several reasons. First, the spectra of neat fats are not readily amenable to accurate quantitative analysis, except at temperatures above the melting point of the fat. Second, prior to the advent of FTIR spectroscopy, IR spectroscopic measurements were generally performed in the transmission mode, and sample thickness was severely restricted by instrumental limitations. In transmission measurements, the IR beam passes through the sample, and only the radiation that is not absorbed by the sample reaches the detector. Because of the low amount of energy produced by mid-IR sources and the high absorptivities of the major absorption bands, very short pathlengths are required to acquire the spectrum of an oil or melted fat in its neat form; otherwise, broad regions of the spectrum are "blanked out" owing to virtually complete absorption of the available energy by the sample. With the detectors employed in dispersive IR spectrometers, the pathlength is further restricted by the limited range of detector linearity (0.15-0.70 absorbance units, corresponding to 20-70% transmission of the energy from the source to the detector) (69). Although a narrow pathlength can be achieved by pressing the sample between two transmission windows, this does not provide the constant pathlength (b in Eq. [2.3]) required for accurate quantitation. Accordingly, for purposes of quantitative analysis without the use of an internal standard, the sample must be injected into a fixed-pathlength transmission cell, consisting of two polished windows, made of salt crystals (e.g., NaCl. KBr), that are separated by a spacer whose thickness determines the pathlength. The extremely short pathlengths (<10 μm) required for the analysis of neat

oils or melted fats with a dispersive IR spectrometer, together with the viscous nature of these types of samples, have made this impractical in the past. However, the higher optical throughput of FTIR spectrometers and the broader range of linearity of the deuterated triglycine sulfate (DTGS) detector commonly employed in these instruments allow somewhat longer pathlengths to be used, making it feasible to record the spectra of oils and melted fats in a transmission cell without dilution (84).

The superior optical throughput and signal-to-noise characteristics of FTIR spectrometers, by comparison with dispersive instruments, have also increased the applicability of a variety of alternatives to transmission-based sample-handling techniques (69). The most important of these has been attenuated total reflectance (ATR), which is based on the optical phenomenon of total internal reflection (85), and much of the work reported in the literature on FTIR analysis of fats and oils has employed a horizontal ATR (HATR) sampling accessory (Figure 2.5). This type of device provides a simple and convenient means of sample handling as oils and premelted fats can be simply poured onto the surface of the ATR crystal and then wiped off after collection of the FTIR spectrum. The ATR technique inherently provides a short effective pathlength, and thus the restrictions on sample thickness that plague measurements in the transmission mode are eliminated. This is because light from the infrared source is not transmitted through the sample but rather is launched into the ATR crystal at an angle such that it undergoes total internal reflection. Under conditions of total internal reflection, an evanescent wave is established at the surface of the ATR crystal whose amplitude decays exponentially with distance from the surface of the crystal. The distance from the surface at which the intensity of the evanescent wave decays to $1/e$ of its value at the surface is

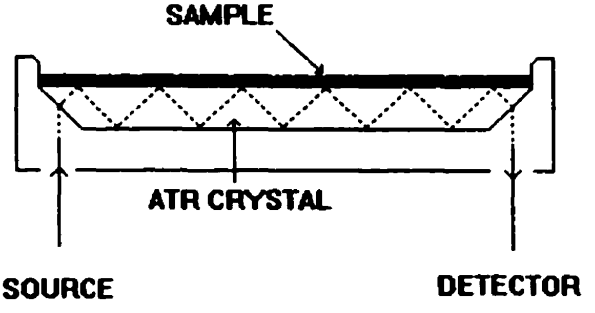


Figure 2.5. Schematic drawing of a multiple-reflection horizontal attenuated total reflectance (HATR) sampling accessory.

defined as the depth of penetration, d_p. The evanescent wave is attenuated by the absorption of radiation by species on or near the surface of the ATR crystal, and measurement of this attenuation as a function of wavelength yields the IR spectrum of these species. The effective pathlength in an ATR measurement is given by the product of the number of internal reflections and the depth of penetration, d_p, given by (85):

$$d_p = \lambda / \{2\pi n_1 [\sin^2(\theta) - (n_2/n_1)^2]^{1/2}\}$$

where λ is the wavelength of the radiation, n_1 is the refractive index of the ATR crystal, n_2 is the refractive index of the sample, and θ is the angle at which the incident light strikes the interface. Because the depth of penetration of the evanescent wave into the sample is only on the order of microns, the band intensities in an ATR spectrum are weak. Most of the work on ATR analysis of fats and oils has involved the use of accessories of the multiple-reflection type (Figure 2.5), in which the totally internally reflected IR beam strikes the surface of the ATR crystal at multiple points, typically 9-11, thereby increasing the effective pathlength. In recent years, single-bounce (SB) HATR accessories have become available that have much higher optical throughput than the multiple-reflection devices and thus yield spectra with a good signal-to-noise ratio despite the very short effective pathlength obtained with only a single reflection. These SB-HATR accessories are particularly useful when only small amounts of sample are available because the surface area of the ATR crystal that must be covered by the sample is much smaller than in the multiple-reflection devices.

Because of their short effective pathlengths, ATR sampling accessories have proved particularly useful in work with strongly absorbing samples such as neat fats and

oils. A flow-through ATR cell has also been shown to have potential utility for on-line monitoring of catalytic hydrogenation processes, without any interference from catalyst particles present in the oil (86). However, the ATR approach to fats and oils analysis has been reported to have a number of drawbacks. These include (i) the general tendency of lipids to strongly adhere to the surface of the ATR crystal (the "memory effect"), which increases the risk of sample cross-contamination, and (ii) the variation in the effective pathlength with slight changes in alignment of the crystal (87, 88). In addition, the inherently short effective pathlength associated with the ATR technique is disadvantageous for applications requiring the detection of species present in oils at low concentrations, such as free fatty acids and oxidation products. For example, in a paper on the determination of free fatty acids (FFAs) by FTIR spectroscopy, the detection limit of FFAs was near 0.2% with an HATR accessory as compared to 0.05% with a 0.1-mm transmission flow cell (89).

2.3.5. Quantitative Analysis Applications

2.3.5.1. Determination of trans Content

At present, the only widespread application of IR spectroscopy in fats and oils analysis is the determination of isolated *trans* isomers, which is an official method of the AOCS, the AOAC (90), and IUPAC (91). *Trans* analysis by IR spectroscopy dates back 50 years (55) and is based on the measurement of the characteristic absorption of isolated *trans* bonds at 966 cm^{-1} (10.3 μm), which is due to their C=C–H out-of-plane bending vibration (Figure 2.6). An implicit assumption of the original method was that fats and oils containing no *trans* double bonds have zero absorbance at the analytical wavelength,

but it was soon established that this is not the case, as all triglycerides exhibit a weak absorption band that overlaps with the *trans* absorption band. The intensity of this overlapping absorption band, which varies with the triglyceride composition of the sample, can contribute as much as 3-5 percentage points to the measured *trans* values (92). Because the underlying triglyceride absorptions will thus cause a substantial overestimation of the *trans* content of samples having low levels of *trans* isomers, the original AOCS method required that samples containing less than 15% isolated *trans* isomers be converted to methyl esters prior to analysis (93). However, although this procedure eliminates the triglyceride absorptions, the *trans* band still sits on a sloping baseline, making accurate measurement of its intensity difficult. Furthermore, when a baseline is drawn between the limits indicated in the original AOCS method, the *trans* values obtained are low by 1.5-3.0 percentage points (92).

The method described above has been widely utilized in the fats and oils industry, particularly in the analysis of partially hydrogenated oils. Because of the importance of this method, it has been the subject of numerous investigations (e.g., Refs. 51, 84, 92, and 94-99), and various improvements of the method have been suggested; much of this work has been reviewed by Firestone and Sheppard (41). Some of the suggested improvements were incorporated when the AOCS method was revised in 1996 (100). The newer method requires the conversion of all samples to methyl esters, regardless of *trans* content. As in the original method, samples are dissolved in CS_2 and their spectra recorded in a fixed-pathlength (1 mm) transmission cell. Separate calibration equations are derived for the analysis of samples containing ≤10% *trans* isomers and >10% *trans* isomers. Instead of

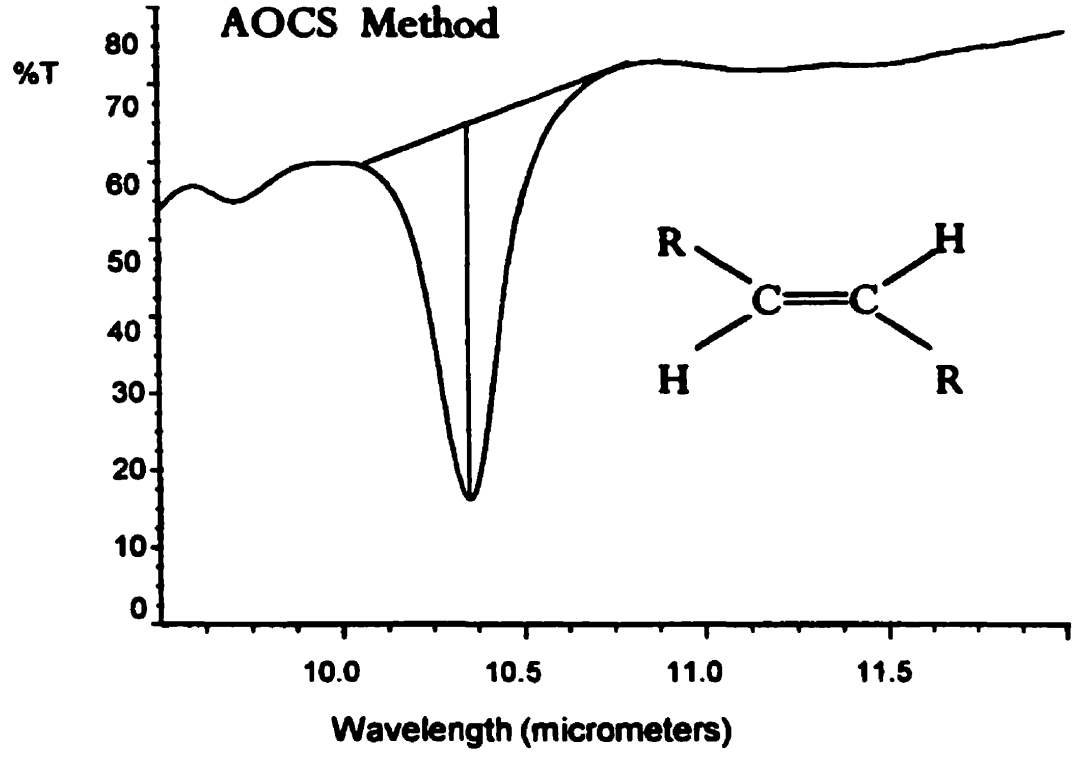


Figure 2.6. Traditional AOCS method for the determination of isolated *trans* isomers in fats and oils by infrared spectroscopy. %T, Percent transmittance.

the single calibration standard employed in the original method (a CS_2 solution of trielaidin or methyl elaidate), a series of standards consisting of mixtures of methyl elaidate and methyl oleate in CS_2 are prepared, with the total concentration of methyl esters kept constant throughout the series. This two-component calibration approach allows for better modeling of the spectral baseline, and hence more accurate *trans* analyses, because the total concentration of methyl esters in each standard and in the samples to be analyzed is the same (0.20 g/10 mL). Other changes in the AOCS method concern the selection of baseline points in the measurement of the *trans* peak height; whereas fixed baseline points were formerly specified, the position at which the baseline is drawn in the modified method depends on the size of the *trans* peak.

Beyond updating the experimental protocol to reflect the data handling capabilities of modern IR spectrometers (both FTIR and dispersive instruments), the modifications of the AOCS method were largely directed toward improving the accuracy of IR *trans* analysis. A number of investigators have also made efforts to simplify the experimental procedure, particularly by analyzing samples in their neat form and thereby eliminating the use of the volatile and noxious/toxic CS_2. For example, Sleeter and Matlock (101) developed an FTIR procedure for measuring the *trans* content of oils, analyzed as neat methyl esters using a 0.1-mm KBr transmission cell. This FTIR method was shown to provide better precision and a significant reduction in total analysis time (2.5 min/sample) in comparison with the traditional AOCS method, as well as having the advantage of being amenable to automation. The method was calibrated with standards prepared by dissolving varying amounts of methyl elaidate in methyl linoleate; the best calibration equation was obtained by a quadratic fit of the *trans* peak area versus

concentration data, owing to a slight curvature in the calibration plot over the concentration range investigated (0-50% *trans*). Sleeter and Matlock reported that more than 700 samples were run in the same cell over a period of nine months without any need for recalibration, demonstrating the stability of the FTIR spectrometer (101). Although this FTIR method greatly simplified the *trans* analysis, particularly by eliminating the need for CS_2, the analysis was still performed on methyl esters rather than neat fats and oils.

A means of eliminating the requirement for conversion to methyl esters is to employ the spectral ratioing capability of FTIR spectrometers to remove the contributions of triglyceride absorptions to the *trans* peak by ratioing the single-beam FTIR spectrum of the fat or oil being analyzed against the single-beam spectrum of a similar reference oil that is free of *trans* groups. In early work with double-beam dispersive spectrometers, the similar approach of placing a CS_2 solution containing a *trans*-free oil in the reference beam to null the triglyceride absorptions in the spectrum of the sample was employed (96), but the FTIR ratioing procedure provides a much simpler and more accurate means of removing these bands from the sample spectrum. In their investigation of the ratioing method, Mossoba *et al.* (102) employed an HATR sampling accessory, which was preheated in an oven when fats were to be analyzed. Calibration was performed using standards prepared by spiking triolein with varying amounts of trielaidin (0.4-44%). The single-beam spectra of these standards were ratioed against the single-beam spectrum of neat triolein, and a calibration plot was obtained by plotting the integrated area of the *trans* band (990-945 cm^{-1}) in these ratioed spectra against concentration. An analogous

procedure was followed with methyl elaidate/methyl oleate mixtures to develop a calibration for use in the analysis of methyl esters. The two calibrations derived were employed in the analysis of partially hydrogenated soybean oils and the corresponding methyl esters, respectively, and yielded similar *trans* values, indicating that the ratioing method adequately compensates for the triglyceride absorptions that overlap with the *trans* band in the spectra of oils (102). The ratioing method, implemented with a heated SB-HATR sample handling accessory, was adopted by the AOCS in 1996 as Recommended Practice Cd 14d-96 for the quantitation of isolated *trans* isomers at levels equal to or greater than 1% (103) and was approved as an AOCS official method in 1999 (104).

Mossoba *et al.* (102) reported that the results obtained by the FTIR ratioing method were strongly dependent on the nature of the reference oil. When analyzing partially hydrogenated soybean oils, they found that the *trans* values obtained by using triolein as the reference oil were 2.6 percentage points higher than those obtained when a refined, bleached, deodorized (RBD) soybean oil served as the reference material. They attributed this difference primarily to the more similar composition of the soybean reference oil to that of the samples being analyzed, making the ratioing out of the overlapping triglyceride absorptions more accurate. This method was subsequently the subject of a limited collaborative study (5 laboratories) (105), in which the test samples were prepared by mixing an RBD soybean oil with an industrial blend or by spiking an RBD oil with trielaidin, and the reference oil was an ultra-degummed, bleached, expeller soybean oil or an RBD soybean oil. In this study, in which the reference oil was well matched to the samples, high accuracy as well as good reproducibility was obtained.

In addition to underlying triglyceride absorptions, other sources of spectral interference in IR *trans* determinations are species having conjugated double bonds, one or more of which has the *trans* configuration. When a *trans* double bond is conjugated with a second double bond, the *trans*-C=C–H bending absorption shifts to higher frequencies by 16-27 cm^{-1}, the magnitude of the shift being dependent on the number and geometric configurations of the conjugated bonds (106). For this reason, the descriptions of the official AOCS methods state that they are not applicable to fats and oils containing large quantities (>0.5%) of conjugated unsaturation, such as tung oil, which contains β-eleostearic acid (18:9*t*,11*t*,13*t*). Conjugated diene hydroperoxides and various secondary oxidation products formed as a result of lipid autoxidation also contain conjugated *trans* double bonds, but, unless oxidation is extensive, the levels of these oxidation products in oils should not be sufficient to cause significant errors in IR *trans* determinations.

When *trans* double bonds are part of a methylene-interrupted double bond system, the position of the *trans* bending absorption is not shifted by comparison with that of *trans*-monoenes, but the extinction coefficient is reduced. Thus, the molar extinction coefficients of mono-*trans*-octadecadienoates and mono-*trans*-octadecatrienoates have been determined to be 0.84 times that of *trans*-octadecenoates, and that of di-*trans*-octadecadienoates is 1.74 instead of 2 times that of *trans*-octadecenoates (107). De Greyt *et al.* (108) pointed out that for the analysis of refined (unhydrogenated) oils, in which the major *trans* isomers are dienoic and trienoic fatty acids having one of their double bonds in the *trans* configuration, calibration standards prepared with trielaidin may not be appropriate. Although this would appear to be a valid point, the experimental results

reported by De Greyt *et al.* do not indicate any significant difference between the performance of a calibration based on refined soybean oils and one based on calibration standards prepared with trielaidin.

In order to evaluate the accuracy of the IR *trans* method, Ratnayake and Pelletier (109) employed the combined procedure of silver nitrate TLC (which affords complete separation of 18:1*t* and 18:1*c* isomers) and capillary GC as the reference method. These authors found that the IR *trans* predictions for margarine samples, analyzed as methyl esters, were about 16% lower than those obtained by the TLC/GC procedure. However, when the IR method was calibrated using a fatty acid methyl ester mixture derived from a partially hydrogenated canola oil, instead of methyl elaidate, the average difference between the IR and TLC/GC methods was only about 3%. Ratnayake and Pelletier (109) attributed this finding to the fact that the traditional calibration approach using a single *trans* isomer (i.e., 18:1Δ9*t*) does not account for differences in IR absorptivity among the various *trans* isomers present in partially hydrogenated vegetable oils (PHVO). These authors thus proposed that for IR *trans* analysis of PHVO, a more suitable approach would be to base the calibration on standards prepared from a well-characterized PHVO.

2.3.5.2. Iodine Value

The possibility of employing IR spectroscopy for the determination of the degree of unsaturation of fats and oils was discussed by Chapman in 1965 (106). In early studies of the IR spectra of unsaturated fatty acids and methyl esters (110), it had been shown that the ratio of the intensity of the =C–H stretching absorption at ~3020 cm^{-1} to the difference between this intensity and that of the CH_2 stretching absorption at 2920 cm^{-1}

was linearly related to the number of *cis* double bonds in fatty acid chains of the same length. Thus, Chapman proposed that measurement of this ratio in the spectra of fats and oils could provide a means of determining unsaturation providing that the samples do not contain appreciable amounts of *trans* isomers, which have much weaker absorption in this region. An IR method for the prediction of IV on the basis of these principles, using a dispersive IR spectrometer, was investigated by Arnold and Hartung in 1971 (111). These authors obtained a linear regression equation relating the iodometrically determined IVs of 25 fats and oils to the absorbance ratio $A(=C-H)/A(CH_2)$ in spectra recorded from carbon tetrachloride solutions of these samples. The IV predictions calculated from this equation for 19 additional samples had an average deviation of <1 IV unit from the iodometrically determined values for these samples. However, the IVs of two samples of hydrogenated soybean oil were underestimated by the IR method by 3.7 and 4.4 IV units, respectively. This result was not unexpected since, as mentioned above, the IR method employed is not applicable to samples containing *trans* isomers. Arnold and Hartung suggested that hydrogenated oils could be analyzed if the standard curve were established using hydrogenated samples of the same type, but they did not investigate this possibility. However, they did conclude that it would not be possible to use a single standard curve to obtain accurate IV predictions for various types of hydrogenated oils. This conclusion is not unreasonable as the IV of oils containing both *cis* and *trans* isomers can be linearly related to the intensity of absorption at a single wavelength only if (i) the *cis* and *trans* isomers have the same extinction coefficient at the measurement wavelength, which is not the case for the =C–H stretching absorption, or (ii) the *cis* and *trans* contents of the

oils are highly correlated. The latter condition would be fulfilled in the case of oils of the same type that are hydrogenated under a particular set of conditions.

Similar results were obtained from an analogous method developed by Anderson *et al.* (112). In this method, differential spectra were recorded by using two matched cells placed in the sample and reference beams of a double-beam dispersive IR spectrometer. Samples were dissolved in carbon tetrachloride at a concentration of 2% (w/w), and the reference was a solution containing an equal concentration of tristearin (18:0). The use of this differential technique improved the resolution of the peak due to the =C–H stretching absorption as the neighboring aliphatic CH stretching absorptions were eliminated to a large extent, and IV was related directly to the intensity of the =C–H stretching absorption by a linear regression equation. Although the authors claimed that this approach simplified the method previously reported by Arnold and Hartung, the latter method had the advantage that it was insensitive to pathlength and concentration because quantitation was based on a peak ratio rather than an absolute intensity.

The above methods were developed with dispersive IR spectrometers and required dissolution of the fat or oil in carbon tetrachloride, as well as manual measurement of peak heights. Total analysis times were reported to be on the order of 20 min. Bernard and Sims (113) developed an analytical protocol that reduced the analysis time by a factor of 10 through the use of a computerized system and by analyzing oils in their neat form in a 0.1-mm cell. Under these conditions, there would be virtually no transmission of infrared energy through the sample in the C–H stretching absorption region, so Bernard and Sims based their IV analysis on the much weaker C=C stretching absorption at about 1660 cm^{-1}. They reported that the calibration equation for this method required an

individual "correlation factor" for each oil type, presumably because the C=C stretching absorption appeared as a shoulder on the ester linkage carbonyl absorption, whose intensity varies as a function of the average molecular weight of the oil.

In the early 1990s, two papers were published concerning the application of FTIR spectroscopy, in conjunction with the ATR sampling technique, in the determination of the unsaturation of fats and oils (88, 114). In both cases, no dissolution of the sample was required because of the short effective pathlength associated with ATR measurements. The FTIR/ATR method described by Afran and Newbery (114) was based on the measurement of unsaturation by the peak-height ratio approach previously used by Arnold and Hartung in their work with a dispersive IR spectrometer. From their analysis of a limited set of samples, consisting of six different natural oils, Afran and Newbery concluded that the results obtained by the FTIR/ATR method showed good correlations with those obtained by both GC and iodometry. These authors did not analyze any hydrogenated oils but, as discussed above, the peak-height ratio approach that they employed would give inaccurate results for such samples.

An entirely different approach was taken by van de Voort *et al.* (88) in the second paper on FTIR/ATR analysis of fats and oils, which reported the development of a method for the simultaneous prediction of both IV and saponification number (SN), a measure of weight-average molecular weight. Instead of employing a simple calibration equation relating the extent of unsaturation to the height of the =C–H stretching absorption or the ratio of the height of this band to that of a CH_2 stretching absorption, these authors adopted the alternative approach of exploiting the powerful capabilities of PLS regression to correlate spectral features to IV as well as SN. Another unique aspect

of this work was the use of a set of 11 pure triglycerides as calibration standards in the development of PLS calibration models for the prediction of IV and SN. This set of standards comprised saturated triglycerides (C8:0, C10:0, ..., C18:0), *cis*-unsaturated triglycerides [triolein (C18:1*c*), trilinolein (C18:2*c*,*c*), and trilinolenin (C18:3*c*,*c*,*c*)], and *trans*-unsaturated triglycerides [trielaidin (C18:1*t*) and trilinolelaidin (C18:2*t*,*t*)]. The use of these pure triglycerides as calibration standards has several advantages. First, the calibrations devised are "universal," in that they are applicable to all refined triglyceride-based oils and fats. Second, this calibration approach has the benefit of eliminating the need for chemical analyses of the calibration standards, as the reference values for the pure triglycerides are known from their molecular structure, and thus the accuracy of the IR method is not limited by the precision of a reference chemical method.

The standard errors of the IV and SN calibrations developed with the use of these pure triglyceride standards were 2.68 IV and 0.93 SN units (88). Validation was performed with 37 fats and oils, varying widely in IV and SN. The FTIR-predicted IV and SN values for these samples were compared with the values obtained by the AOCS official methods for the determination of these two parameters. The results indicated that IV and SN can be determined with 95% confidence by the FTIR/ATR method to within 2.8 and 2.0 units, respectively, of their chemically determined values (88).

For the analysis of both fats and oils by this FTIR/ATR method, a germanium ATR crystal was heated to 60°C by attaching four 25-W strips of heating tape onto the metal plate supporting the crystal; the temperature of the sample was monitored by a probe placed in direct contact with the sample and was controlled to within ±0.2°C. After

analysis of each sample, the ATR crystal was cleaned with a 1% Triton X-100 solution and with hexane and then allowed to dry. The resulting evaporative cooling of the ATR crystal was found to be an important source of analytical error because the effective pathlength of an ATR cell depends, in a wavelength-dependent manner, on the refractive indices of the ATR crystal and the sample, which are, in turn, temperature-dependent. A change in temperature by more than 1°C was observed to result in a substantial change in the curvature of the spectral baseline, which affected the analytical reproducibility of the method. Other sources of error associated with the ATR sampling technique were the general tendency of lipids to strongly adhere to the surface of the ATR crystal (the "memory effect"), increasing the risk of sample cross-contamination, and the variation in effective pathlength with slight changes in the alignment of the ATR sampling accessory.

Several groups have investigated the potential utility of Raman spectroscopy as a technique for the determination of IV. Raman spectroscopy is a complementary technique to IR spectroscopy, in that both techniques measure molecular vibrational transitions, but the intensities of the bands due to these vibrational transitions in IR and Raman spectra are governed by different selection rules, such that bands that are intense in IR spectra tend to be absent or weak in Raman spectra, and vice versa. Thus, the Raman spectra of unsaturated fatty acids in the region between 1750 and 1600 cm^{-1} are dominated by a strong band due to the C=C stretching vibration which can be measured with minimal interference from the weak ester linkage carbonyl absorption (115), whereas the opposite pattern of relative intensities is observed in IR spectra. The strength of the C=C stretching absorption in Raman spectra makes it potentially suitable for use in the determination of

IV. This possibility was initially investigated with a traditional Raman spectrometer employing visible laser excitation (115). In more recent work, FT-Raman spectroscopy with near-IR laser excitation was employed in order to avoid background fluorescence resulting from electronic excitation of colored components present in oils and margarines by the Raman exciting light (116). Good-quality spectra were acquired from oils in glass bottles and margarines smeared into a solid sample holder with a 4-min acquisition time. For the oils, a plot of the ratio of the peak height at 1661 cm^{-1} to that at 1444 cm^{-1} (CH_2 scissoring vibration) versus IV was linear, with a correlation coefficient of 0.9987. In the case of margarines, the corresponding plot gave a much poorer correlation (r = 0.9495), but use of peak areas instead of peak heights increased the correlation coefficient to 0.9899. This result was attributed to the fact that the C=C stretching band of *trans* isomers, which were present in the margarines but not to a significant extent in the oils, is shifted to higher frequency by 10 cm^{-1} as compared to that of *cis* double bonds. Thus, the contribution of *trans* isomers to IV was measured only when peak area (1694-1628 cm^{-1}) was used. The authors of this study suggested that, with the use of optical fibers, FT-Raman spectroscopy may serve in the future as a technique for on-line monitoring of changes in IV during hydrogenation processes (116).

2.3.5.3. Free Fatty Acids

Oils containing free fatty acids (FFAs) exhibit a ν(C=O) absorption due to FFA dimers at 1711 cm^{-1}. This band is difficult to measure accurately because it appears as a shoulder on the intense ester linkage carbonyl absorption band; at low FFA levels, the FFA band may not even be discernible. However, two methods of compensating for this

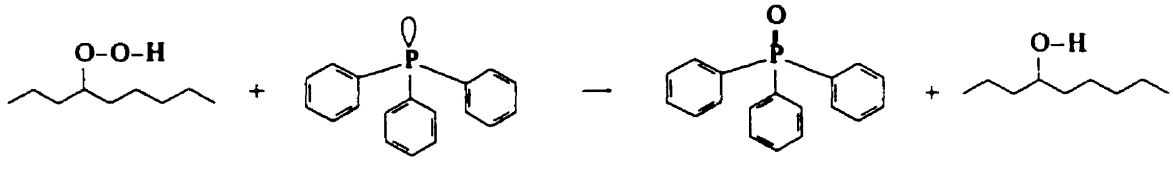
band overlap in the determination of FFAs by FTIR spectroscopy have been proposed in the literature (89, 117). Lanser *et al.* (117) employed spectral deconvolution to sharpen the FFA band and diminish its overlap with the ester carbonyl absorption. Using calibration standards prepared by spiking an RBD soybean oil with oleic acid and spanning a range of 0-5% FFA, they constructed a calibration curve relating FFA content to the peak area of the 1711-cm^{-1} band. The accuracy of this method in measuring the elevated levels of FFAs that are present in crude oils extracted from damaged soybeans was assessed with 19 samples; the FTIR-predicted values of FFA content were found to be within 0.5 percentage points of the values obtained by the AOCS titration method. In another study, Ismail *et al.* (89) eliminated the spectral interference due to the ester carbonyl absorption by ratioing the spectrum of the sample against the spectrum of an FFA-free oil of the same type. These authors demonstrated that FFA levels down to 0.05% could be detected after application of this spectral ratioing technique. However, accurate FFA analyses could not be obtained for oils that had undergone thermal or oxidative stress owing to the presence of various carbonyl-containing species (aldehydes and ketones) that spectrally interfere with the measurement of the FFA absorption band. For such samples, an indirect method was developed, involving extraction of the FFAs in 1% KOH/methanol and quantitation of the carboxylate anion by measurement of the $\nu(COO^-)$ absorption band at 1570 cm^{-1}. Both the direct and the indirect method were shown to be comparable in precision and accuracy to the AOCS titration method (89).

Recently, Bertran *et al.* (118) reported a PLS-based FTIR method for the determination of FFAs designed specifically for the analysis of olive oils of different types and origins. The optimum spectral region for the analysis was found to be 1775-

1689 cm^{-1}. Individual PLS calibration models were developed for two concentration ranges (0.1-0.5% and 0.5-2.1% FFA), using absorbance, first- and second-derivative, and standard-normal-variate (SNV)-corrected spectra. The latter pretreatment of the spectral data was reported to yield better predictive accuracy when thermally stressed samples were analyzed with the use of calibration models based on fresh oils.

2.3.5.4. Measurements of Oxidative Status

2.3.5.4a. Determination of Peroxide Value. Several approaches to determining PV by FTIR spectroscopy have been investigated by the McGill IR Group (119-122). The first approach examined was based on the results of the work reported in Chapter 3 of this thesis and entailed measurement of the hydroperoxide OO–H stretching absorption (119). A PLS calibration model was developed to account for spectral interferences due to other OH-containing species that may be present in oils, such as alcohols, mono- and diglycerides, FFAs, and water, all of which exhibit O–H stretching absorptions that overlap with the hydroperoxide band. Owing to the inherent complexity of this approach, which made it impractical to implement, a simpler means of quantitating hydroperoxides was subsequently developed (120). This method is based on the rapid reaction between hydroperoxides and excess triphenylphosphine (TPP), which leads to the formation of triphenylphosphine oxide (TPPO) in stoichiometric amounts:



TPP TPPO

This reaction had previously been successfully employed by Nakamura and Maeda (123)

in a microassay for lipid hydroperoxides in biological samples, using a combination of HPLC and UV detection. The utility of this reaction for the determination of PV by FTIR spectroscopy is dependent on the ability to accurately quantitate TPPO in the presence of TPP. This proved to be readily achievable owing to the presence of a unique and sharp band at 542 cm^{-1} in the spectrum of TPPO, which is assigned to an X-substituent-sensitive phenyl vibration (124); the corresponding band in the spectrum of TPP is broad and shifted ~40 cm^{-1} to lower frequency. A calibration equation relating PV to the height of the TPPO band at 542 cm^{-1}, measured relative to a single baseline point at 550 cm^{-1}, was derived by simple linear regression (R = 0.999, SD = 0.056 PV) (120). The method was validated by analyzing both oxidized oils and oils spiked with *t*-butyl hydroperoxide. A detection limit of 0.10 PV and excellent reproducibility (±0.18 PV) were obtained. The standardized analytical protocol developed for this PV method consisted of adding ~0.2 g of a TPP/hexanol stock solution (33% w/w) to ~30 g of melted fat or oil, shaking the sample, and scanning its spectrum in a 0.1-mm KCl transmission cell maintained at 80°C, the total analysis time being about 2 min/sample. The suitability of using disposable polyethylene IR cards as a convenient alternative means of sample handling was subsequently demonstrated, and the possibility of preimpregnating the cards with the TPP reagent was examined (121). The principles of this PV method were also employed in the development of a PLS-based FT-near-IR method (122).

2.3.5.4b. Determination of Anisidine Value. Aldehydes exhibit strong bands in the 1730-1680 cm^{-1} region of the IR spectrum, owing to the high absorptivity of their C=O stretching vibrations, with saturated, α,β-unsaturated, and α,β,δ,γ-unsaturated aldehydes

being distinguishable (125). On the basis of the ability to distinguish between these three classes of aldehydes, Dubois *et al.* (125) formulated a synthetic calibration approach to the FTIR determination of AV based on the results of the work presented in Chapter 3 of this thesis. A PLS calibration model for the prediction of the concentrations of the three aldehyde classes was developed by using a set of 32 synthetic calibration standards prepared by spiking canola oil with varying amounts of hexanal, *trans*-2-hexenal, and *trans,trans*-2,4-decadienal, as well as with random amounts of other compounds representative of oxidation by-products in order to model potential spectral interferences in oxidized oils. The calibration standards employed in developing the PLS calibration model were analyzed by the AOCS AV method, and a relationship between the chemically determined AV and the gravimetrically added amounts of the three aldehydes was obtained by multiple linear regression (125). This linear regression equation was then employed to convert the PLS-predicted aldehyde concentrations of thermally stressed canola oil samples to "apparent" AV. Regression of the "apparent" AV values obtained against the chemically determined AV values of these samples yielded a standard error of prediction of 1.65 AV units. Similar predictive accuracy was obtained from an alternative calibration approach based on the use of thermally stressed oils as calibration standards (125). As such, quantitative determination of AV by FTIR spectroscopy was shown to be feasible, and the synthetic calibration approach provided additional information on the aldehyde types present in a sample. This study provided the basis for the development of a rapid, automated FTIR method for AV analysis of thermally stressed fats and oils in their neat form without the use of chemical reagents, with possible application in the monitoring of the oxidative state of frying oils.

2.3.5.5. Solids Content of Fats

The solids content of fats as a function of temperature has an important bearing on the functional characteristics of margarines, shortenings, and other fat blends, and thus solids content is an important QC parameter in the edible fats and oils industry. In North America, solids content determinations have traditionally been performed using dilatometry to obtain the solid fat index (SFI), an empirical measure of the change in the specific volume of the fat as a function of temperature (126). In Europe, solid fat content (SFC), based on NMR spectroscopy, has been widely used as the preferred method of determining fat solids, and it has recently been approved as an official method by the AOCS (127). Both the SFC and SFI procedures involve measurements at a series of set temperatures and are fairly lengthy because tempering of the sample at each temperature is required in order to obtain reproducible values.

Van de Voort *et al.* (128) proposed an FTIR method that allows a four-temperature SFI profile of a fat to be predicted from a single FTIR measurement on the neat, melted sample. The underlying principle of this method is that the SFI or SFC profile of a fat is defined by its fatty acid composition and distribution, which in turn is characterized by the IR spectrum of the melted fat, as the spectrum represents the superposition of all the contributions of the individual triglycerides making up the fat. Calibration of the method was based on the development of individual PLS calibration models relating the spectral features of melted fat samples to their known dilatometric SFI values obtained at each of the four common temperatures of measurement (50, 70, 80, and 92°F). By employing this approach, an FTIR SFI method was developed using 72 samples of partially hydrogenated soybean oil from 11 hydrogenation runs, obtained from

a major vegetable oil processor and preanalyzed for SFI by the AOCS dilatometric method. Half of these samples were employed as calibration standards, with the remainder serving as validation samples. Based on duplicate analyses of the validation samples one week apart, the accuracy and reproducibility of the FTIR SFI method were determined to be ±0.60 and ±0.38 SFI units, respectively (128). Thus, it was concluded that the FTIR method has the potential to serve as a substitute for the traditional dilatometric method for the determination of solids content, with the advantage of a reduction in the analysis time from hours to minutes due to the elimination of the tempering steps required in the dilatometric method. One of the limitations of the FTIR approach to the determination of solids content is that the calibration models derived are only applicable to samples with similar characteristics to those of the standards used to derive the calibration, making it necessary to develop a separate calibration for each type of oil or blend.

2.3.5.6. Concluding Remarks

The publications that have been cited in this section encompass the major developments in quantitative IR analysis of fats and oils up to the end of 1999. A number of additional publications have appeared in which these developments have served as the basis for methods designed for specific products. In particular, extensive work has been done in Malaysia on the development of FTIR methods for the analysis of palm oil and palm olein based on the principles of some of the methods reviewed above (129-133).

Apart from the work on quantitative analysis methods considered in this review of the literature, several authors have investigated the potential utility of FTIR spectroscopy

in the authentication of oils and the detection of adulteration, through the use of multivariate statistical methods, such as principal component analysis and discriminant analysis (134-137). In addition, the applicability of FTIR methods developed for the analysis of processed fats and oils to the characterization of fats extracted from food products has been investigated to a limited extent. For example, Liescheski (138) examined the possibility of using supercritical fluid extraction coupled to IR spectroscopy (SFE-IR) to determine the IV of fats in foods. The applicability of the SB-HATR method for the determination of *trans* isomers, described in Section 2.3.5.1, to the analysis of the *trans* fatty acid content of food products has also been examined by researchers at the U.S. FDA, in anticipation of amendments to the nutrition labeling requirements with respect to *trans* fat (139). As mentioned in Chapter 1, amendments were proposed in late 1999 that would require the inclusion of *trans* fatty acids in the amount stated for saturated fat content on nutrition labels, together with a footnote reporting the *trans* fatty acid content. If these amendments are adopted, it may be anticipated that the SB-HATR method will find extensive use, and this in turn should provide an impetus for the wider application of FTIR analytical methodology in the industrial sector.

It may be noted from the review of the literature presented above that the McGill IR Group has taken a leading role in the development of FTIR methods for the analysis of edible fats and oils. The research described in the following chapters of this thesis was begun in the early stages of this analytical methodology development program and established the underlying principles for some of the methods that have been described in this chapter.

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CHAPTER 3

MONITORING THE OXIDATION OF EDIBLE OILS BY FTIR SPECTROSCOPY

3.1. ABSTRACT

Edible fats and oils in their neat form are ideal candidates for Fourier transform infrared (FTIR) analysis, in either the attenuated total reflectance (ATR) or the transmission mode. FTIR spectroscopy provides a simple and rapid means of following complex changes that take place as lipids oxidize. Safflower and cottonseed oils were oxidized under various conditions, and their spectral changes were recorded and interpreted. The critical absorption bands associated with common oxidation end products were identified by relating them to those of spectroscopically representative reference compounds. The power and utility of FTIR spectroscopy to follow oxidative changes was demonstrated through the use of "real-time oxidation plots." A quantitative approach is proposed in which standards that are spectroscopically representative of oxidative end products are used and the oxidative state of an oil is defined in terms of percent hydroperoxides, percent alcohols, and total carbonyl content. By using either relative absorption as a basis or calibrating on representative standards, FTIR analysis provides a rapid means of evaluating the oxidative status of an oil or of monitoring changes in oils undergoing thermal stress.

3.2. INTRODUCTION

Autoxidation is a major deteriorative reaction affecting edible fats and oils and is a primary concern to processors and consumers from a quality standpoint, because the

breakdown products cause marked off-flavors in an oil. Although relatively well understood in general terms, autoxidation is quite complex and variable, depending on oil type and conditions of oxidation (1). A wide range of end products are associated with the autoxidative deterioration of oils at ambient temperatures (2-5), the most important being hydroperoxides, alcohols, and aldehydes. Moisture, hydrocarbons, free fatty acids (FFAs) and esters, ketones, lactones, furans, and other minor products may also be produced, with the FFAs becoming more important in thermally stressed oil. In addition, there is significant *cis* to *trans* isomerization and conjugation of double bonds in the hydroperoxides formed as an oil oxidizes. The rate of oxidation and the distribution of accumulated products depend strongly on the oil source, fatty acid composition, degree of unsaturation, presence of metal ions and antioxidants, time, and thermal stress, making it essential to have a reliable means of assessing the oxidative status and of forecasting the oxidative stability of an oil. The AOCS has a number of official methods to provide an indication of the oxidative status of an oil (6), the most common being the peroxide value (PV), the anisidine value (AV) and the thiobarbituric acid (TBA) test. These chemical methods, which are largely empirical in nature, measure either the primary or secondary products of oxidation, i.e., hydroperoxides or carbonyl-type compounds.

Our research seeks to develop rapid, general-purpose FTIR-based quality control methods for the food industry (7-11). Recently, we have focused on developing simple, rapid methods for oil analysis, as oils in their neat form are ideal candidates for FTIR applications. Methods for the determination of iodine value (12), saponification number (12), and FFAs (13) have been developed, and a number of other methods are in the development stage (PV and AV). The rationale for turning to a new version of an old

technology (IR) is that FTIR spectrometers have many advantages over conventional dispersive instruments, including more energy throughput, excellent wavenumber reproducibility and accuracy, extensive and precise spectral manipulation capabilities (ratioing, subtraction, derivative spectra, and deconvolution), and advanced chemometric software to handle calibration development (7). Because of these advantages, FTIR spectroscopy can provide much more information on the characteristics, composition, and/or chemical changes taking place in fats and oils than can be obtained from conventional dispersive IR instruments. Furthermore, from a practical viewpoint, FTIR quantitative analysis methods are generally very rapid (1-2 min), can be automated, and reduce the need for solvents and toxic reagents associated with wet chemical methods for fats and oils analyses, making the development of FTIR methods timely in view of present efforts to eliminate toxic solvents (14).

Neat oils applied directly onto a multiple-reflection attenuated total reflectance (ATR) crystal or pumped through a transmission flow cell provide high-quality spectra. Solid fats can be handled analogously with an accurately thermostated (~±0.2°C) ATR crystal or flow cell heated to a temperature above the melting point of the fat. Fats and oils, being relatively simple molecular systems composed primarily of triglycerides, exhibit fairly uncomplicated spectra. Most of the important absorption bands lie over the range of ~3500 to 800 cm^{-1}, a region where most of the more rugged transmission window/ATR materials transmit.

The overall objective of the present work is to lay the foundation for the development of FTIR methods for assessing oil quality in relation to lipid oxidation and

thermal stress (e.g., frying oils). A prerequisite to using FTIR spectroscopy to assess the various end products of lipid oxidation is a basic understanding of the spectral changes that take place as an oil oxidizes. Fundamental IR characterization and identification of specific products formed from the oxidation of individual fatty acids and the decomposition of fatty acid hydroperoxides has been done (15, 16), but there have been no comprehensive FTIR spectroscopic investigations of edible oils undergoing oxidation. Thus, there is a need to characterize the spectral changes occurring as oil oxidation proceeds, assign wavelengths to the more common molecular species produced, and assess potential spectral interferences so that a generalized approach to monitoring and quantitating key products associated with oxidation can be developed. This paper describes the FTIR spectral techniques used to follow the main chemical events occurring as oils oxidize, develops a means by which such events can be tracked on a relative basis, and lays the foundation for a quantitative approach to measuring lipid oxidation.

3.3. EXPERIMENTAL PROCEDURES

Instrumentation. The instrument used for this work was a Nicolet 8210 FTIR spectrometer (Nicolet Instrument Inc., Madison, WI) run under a DX operating system (17). To minimize water vapor and CO_2 interferences, the instrument was purged with air from a Balston dryer (Balston, Lexington, MA). A heated horizontal ATR sampling accessory equipped with 40° and 45° ZnSe crystals and a 90-μm CaF_2 flow cell were used in this study. All spectra were collected by co-adding 512 scans at a resolution of 4 cm^{-1} and a gain of 2.0. For oil oxidation monitoring experiments, the Nicolet spectrometer was programmed with macro-command language (12) to automatically record spectra at specified time intervals, abstract absorbance values (peak height) at

preselected frequencies, and print the results for each spectrum recorded. The time/absorbance data matrix was then entered into a standard graphics program to produce "real-time oxidation plots."

Samples/Oxidation. Commercial safflower and cottonseed oil, obtained from a local retail outlet, were used as the base materials for the oxidation studies. Fifty grams of each oil was poured into a 500-mL fritted funnel, packed with activated silica gel (60-200 mesh; Aldrich, Milwaukee, WI), and eluted with 200 mL of hexane to remove any oxygenated compounds from the oil, and the solvent was removed from the eluent on a rotary evaporator under reduced pressure. The oil purified by this procedure was used as a reference oil. The efficiency of this procedure in removing hydroperoxides and carbonyl compounds was assessed by carrying out the PV and AV tests (6) on the unpurified and purified oils; PV was reduced from 2-6 to <1.0 and AV from 2-3 to <1.0 by the purification step. An FTIR procedure was devised to test for the presence of residual alcohols. For this test, 100 μL of deuterium oxide (D_2O) was added to a 2-mL aliquot of the purified sample to exchange any OH groups, thereby shifting their absorptions to lower frequency; ratioing the spectrum of the untreated portion of the oil against that of the D_2O-treated portion then allowed the hydroxyl absorptions in the spectrum of the untreated oil to be clearly observed, without spectral interference from the absorptions of the base oil.

Two sets of oxidation experiments were carried out, one at lower temperatures (70-75°C) with a heated ATR crystal and another at higher temperatures (100, 130, and 230°C) with a CaF_2 flow cell. For the ATR work, 1 mL of a fresh oil sample was pipetted onto the crystal (surface area ≈ 8 cm^2), and its single-beam spectrum was

recorded and ratioed against a single-beam spectrum of air (clean crystal surface). Single-beam spectra were subsequently recorded at 0.5-h intervals from this sample as it oxidized on the ATR crystal and were automatically ratioed against the single-beam spectrum of the fresh oil recorded at t = 0. For the higher-temperature oxidation, a reservoir of oil (~100 mL) was placed in a heating mantle, with the oil temperature adjusted with a rheostat. A positive-displacement piston pump (FMI Lab Pump Model QG50, Oyster Bay, NY) was used to circulate the oil (~3 mL/min) from the reservoir through the CaF_2 flow cell. A single-beam spectrum of the oil was collected as soon as the temperature of the reservoir equilibrated to operating temperature (defined as t = 0). Subsequently, dry air was bubbled continuously through the oil at a rate of ~3 mL/min, and single-beam spectra were recorded every 0.5 h and ratioed against the spectrum recorded at t = 0.

Oxidation Reference Spectra. Commercially available olive oil was spiked with compounds selected as spectroscopically representative of primary and secondary products of oxidation, including (a) oleic acid, (b) *t*-butyl hydroperoxide, (c) hexanal, (d) *trans*-2-hexenal, (e) *trans,trans*-2-4-decadienal, (f) hexanol, and (g) *trans*-4-hexen-3-one (Aldrich Chemicals, Milwaukee, WI), all added at a level of 2% (w/w). A water-saturated sample (~0.02%) was prepared by adding 1% water to olive oil, shaking, and centrifuging (~10,000 × *g*) to remove residual free water. Spectra of these spiked samples were recorded on a 40° ATR crystal at room temperature and ratioed against the spectrum of the unspiked olive oil to develop a spectral library. This library was used to assign the characteristic absorption bands of key functional groups of compounds that may accumulate in oils undergoing oxidation.

3.4. RESULTS AND DISCUSSION

3.4.1. The Concept of Spectral Ratioing

One of the fundamental strengths of FTIR spectroscopy lies in its ability to accurately ratio spectra, allowing one to see small differences that normally might not be apparent in the raw spectrum. The concept of spectral ratioing arises in FTIR spectroscopy because most FTIR spectrometers are single-beam instruments. Thus, the single-beam spectrum recorded for a sample (I_S) consists of the emittance spectrum of the source on which are superimposed the absorptions of the sample as well as of air in the optical path. To eliminate the contributions of the source and the air background, the single-beam spectrum of the sample is digitally ratioed against a single-beam spectrum recorded with no sample in the beam (I_0), yielding the absorbance spectrum of the sample [$A_S = -\log(I_S/I_0)$]. Alternatively, the single-beam spectrum of the sample may be ratioed against the single-beam spectrum of a reference (I_R), thereby eliminating the spectral features common to the sample and the reference in addition to the contributions from the source and air background. This operation [$A_S^r = -\log(I_S/I_R)$] is equivalent to a 1:1 subtraction of the reference spectrum ratioed against an air background from the sample spectrum ratioed against the same background ($A_S - A_R$) but requires only a single mathematical manipulation, i.e.,

$$A_S - A_R = -\log(I_S/I_0) - [-\log(I_R/I_0)] = -\log(I_S/I_R) = A_S^r \qquad [3.1]$$

Figure 3.1a illustrates the absorbance spectrum of olive oil in its neat form on an ATR crystal, obtained by ratioing the single-beam spectrum of the oil against the single-beam spectrum of air (bare crystal surface). This spectrum illustrates the dominant

spectral features associated with edible oils: the CH stretching absorptions in the region from 3050 to 2800 cm^{-1} (*cis*-C=CH, CH_2, CH_3, and CH_2/CH_3 stretching bands), the carbonyl absorption of the triglyceride ester linkage at 1744 cm^{-1}, and the bands associated with the fingerprint region (1500-1000 cm^{-1}). In the spectra of hydrogenated oils, an additional strong band is observed at ~965 cm^{-1} due to the C=C–H bending vibration of *trans* double bonds; measurement of this absorption band in the IR spectrum is the basis for the AOCS official method for the determination of isolated *trans* isomers (6). Figure 3.1b is the spectrum of the same oil spiked with 0.5% *trans,trans*-2,4-decadienal. In this spectrum it is difficult to detect the presence of this contaminant by simple inspection. Upon ratioing the spectrum of the spiked sample against that of the unspiked oil (Figure 3.1c), the spectral features of decadienal became quite apparent (bands in the regions 1750-1600 and 1200-900 cm^{-1} as well as two weak bands in the 2850-2700 cm^{-1} region), as the spectral contributions of the oil have been ratioed out, leaving a clear spectral window that allows the differences between the two oil samples to be detected.

The ratio technique is a very sensitive means of detecting relative spectral changes as long as the signal reaching the detector is strong enough to be measured accurately. For example, for regions of intense absorption in Figure 3.1a (i.e., the triglyceride ester linkage and major CH bands, where absorbance values are >2.0 absorbance units), the signal reaching the detector is too small to be sampled properly, leading to digitization noise in the ratioed spectrum, as seen in Figure 3.1c at ~3000-2800 and ~1750 cm^{-1}. This problem can be avoided by reducing the effective pathlength of the cell so that such bands are not as intense and will ratio out, but at the expense of sensitivity in other

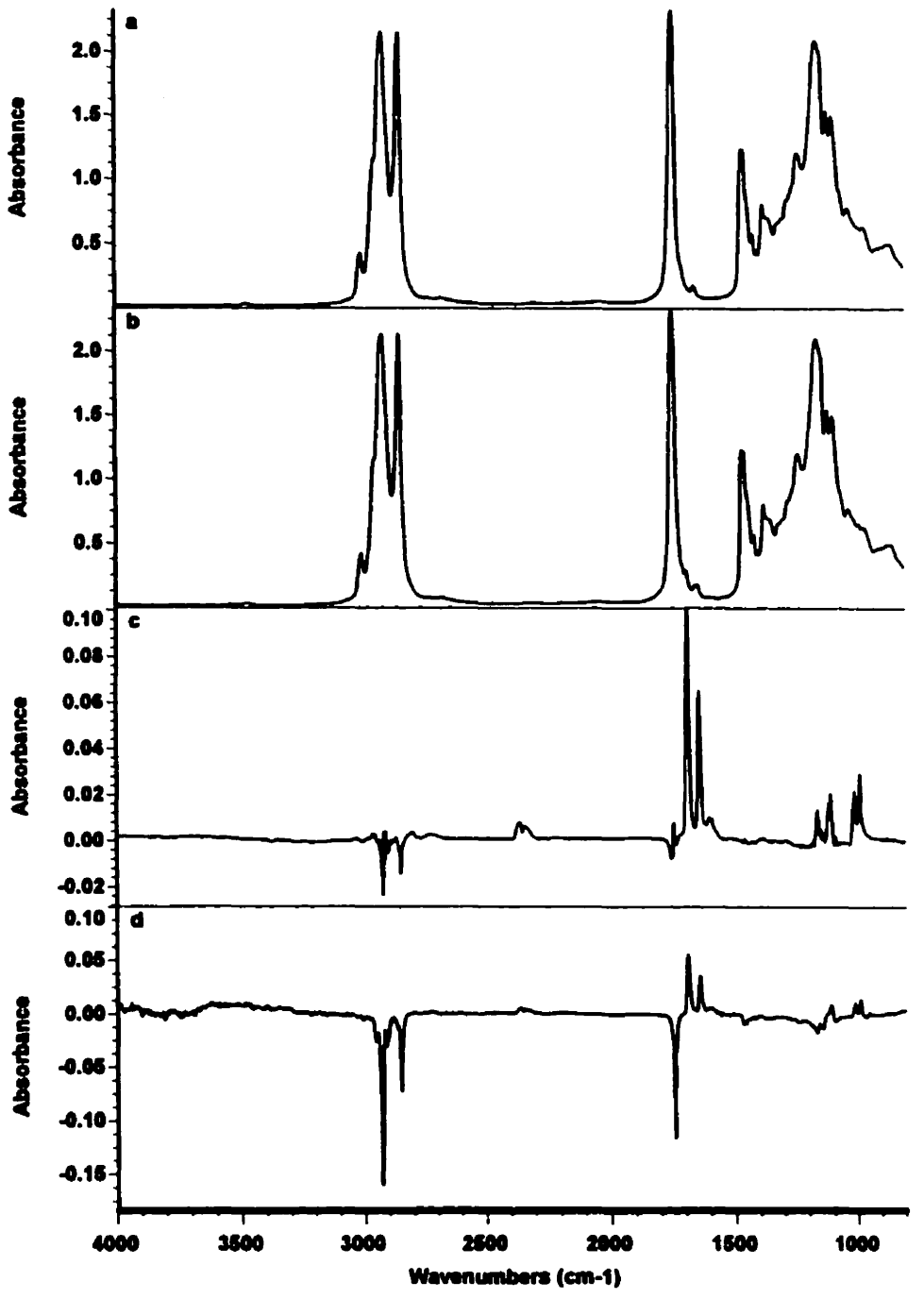


Figure 3.1. ATR/FTIR spectra of pure olive oil (a) and olive oil spiked with 0.5% 2,4-*trans*,*trans*-decadienal (b) on a 40° ZnSe crystal, the difference spectrum obtained by ratioing the single-beam spectrum of the spiked sample against that of the pure olive oil (c), and the corresponding difference spectrum for a 45° ZnSe crystal (d).

regions. This is illustrated by comparing Figures 3.1c and 3.1d, the ratioed spectra obtained for the same spiked sample on a 40° and a 45° ZnSe crystal, respectively, the latter having a lower depth of penetration and, hence, a shorter effective pathlength. In Figure 3.1d, negative peaks are observed in the CH stretching region and at the position of the triglyceride ester linkage absorption, whereas digitization noise is observed in these regions in Figure 3.1c. These negative peaks arise because of the dilution effect produced by spiking, such that the oil absorptions are more intense in the spectrum of the unspiked oil than in that of the spiked sample. Owing to the lower depth of penetration of the 45° crystal, the spectral features of *trans,trans*-2,4-decadienal are clearly much weaker in Figure 3.1d than in Figure 3.1c, with the weak bands in the 2850-2700 cm^{-1} region not discernible. In general, unless measurements are specifically required in regions of high absorption, it is best to use a longer pathlength and benefit from the resulting increased sensitivity. The ability to routinely carry out spectral ratioing (single-beam spectra) and/or its equivalent, spectral subtraction, in the case of absorbance spectra, in an accurate and reproducible manner is a key element in our approach to developing FTIR-based methods for oil analysis.

3.4.2. Analysis of Oxidative Reference Compounds

A spectral library was prepared by recording the spectra of compounds having functional groups representative of common oil oxidation products. Alcohols, saturated aldehydes, and α,β-unsaturated aldehydes, which have been reported as major secondary oxidation products (2, 4), were all represented in the spectral library by C_6 homologues, with α,β,δ,γ-unsaturated aldehydes represented by *trans,trans*-2,4-decadienal, a major decomposition product from heated oxidized linoleate (4). *t*-Butyl hydroperoxide was

selected to represent hydroperoxides because of its stability. Although ketones are minor products in oxidized oils, a C_6 allylic ketone was included in the spectral library to investigate the extent of overlap between the aldehyde and ketone absorptions. Oleic acid and water were also included in view of their possible presence in oils, particularly under hydrolysis conditions. Although the literature abounds with spectral assignments for compounds of all these types (18, 19), it was necessary to obtain spectral data for these compounds in oil to account for any spectral shifts that might occur in this medium (e.g., as a result of hydrogen bonding). Table 3.1 tabulates the functional group frequencies determined by an analysis of these reference spectra and gives their relative absorptivities in relation to the strongest absorbing functional group (ν C=O of hexenal = 1.0).

Table 3.1 shows that each of the various types of oxidation products gives rise to discernible and characteristic absorptions in the FTIR spectrum. Some comments are warranted with respect to the positions of these absorptions. Figure 3.2 presents the OH stretching region in the water, *t*-butyl hydroperoxide, hexanol, and oleic acid reference spectra, plus the composite spectrum obtained by co-adding these spectra. Figure 3.2a-d illustrates that the hydroxyl absorptions of all these compounds occur in the same vicinity (3800-3100 cm^{-1}) and are inherently broad due to hydrogen bonding. Figure 3.2e, the composite spectrum, illustrates the extensive overlap that occurs when all these compounds are present simultaneously in equal amounts. Even with this overlap, most peaks, except for the hexanol band and the lower-frequency component of the water band, appear to be sufficiently separated to enable identification. The presence of alcohol can be established by comparing the intensity of the water/alcohol overlapping band to that of

Table 3.1. Peak Positions and Relative Strengths of the Functional Group Absorptions of Reference Compounds Representative of Products Formed in Oxidized Oils Obtained on a 40° ZnSe ATR Crystal

Compound	Vibration[a]	Peak maximum (cm^{-1})	Relative absorptivity[b]
Water	ν ***OH***	3650 & 3550	NA
	δ ***HOH***	1625	NA
Hexanol	ν R***OH***	3569	0.06
t-Butyl hydroperoxide	ν RO***OH***	3447	0.04
Hexanal	ν R***HC***=O	2810 & 2712	0.02 & 0.03
	ν RH***C=O***	1727	0.20
Hexenal[c]	ν R***HC***=O	2805 & 2725	0.03 & 0.03
	ν RH***C=O***	1697	1.00
	ν R***C***=***C***H-HC=O	1640	0.10
	δ R***C***=***C***H-HC=O	974	0.25
2,4-Decadienal[c]	ν R***HC***=O	2805 & 2734	0.03 & 0.02
	ν RH***C=O***	1689	0.81
	ν R***C***=***C***H-HC=O	1642	0.52
	δ R***C***=***CH***-HC=O	987	0.27
4-Hexen-3-one[c]	ν R***C(=O)***HC=CHR	1703 & 1679	0.23 & 0.38
	ν RC(=O)H***C***=***C***HR	1635	0.32
	δ RC(=O)H***C***=***CH***-R	972	0.29
Oleic acid	ν RCO***OH***	3310	0.03
	ν R***C(=O)***OH	1711	0.25

[a]The bonds of each functional group that are responsible for the absorption band(s) are presented in bold/italic type.

[b]Relative to a value of 1 for the strongest absorption band.

[c]All double bonds in the *trans* form.

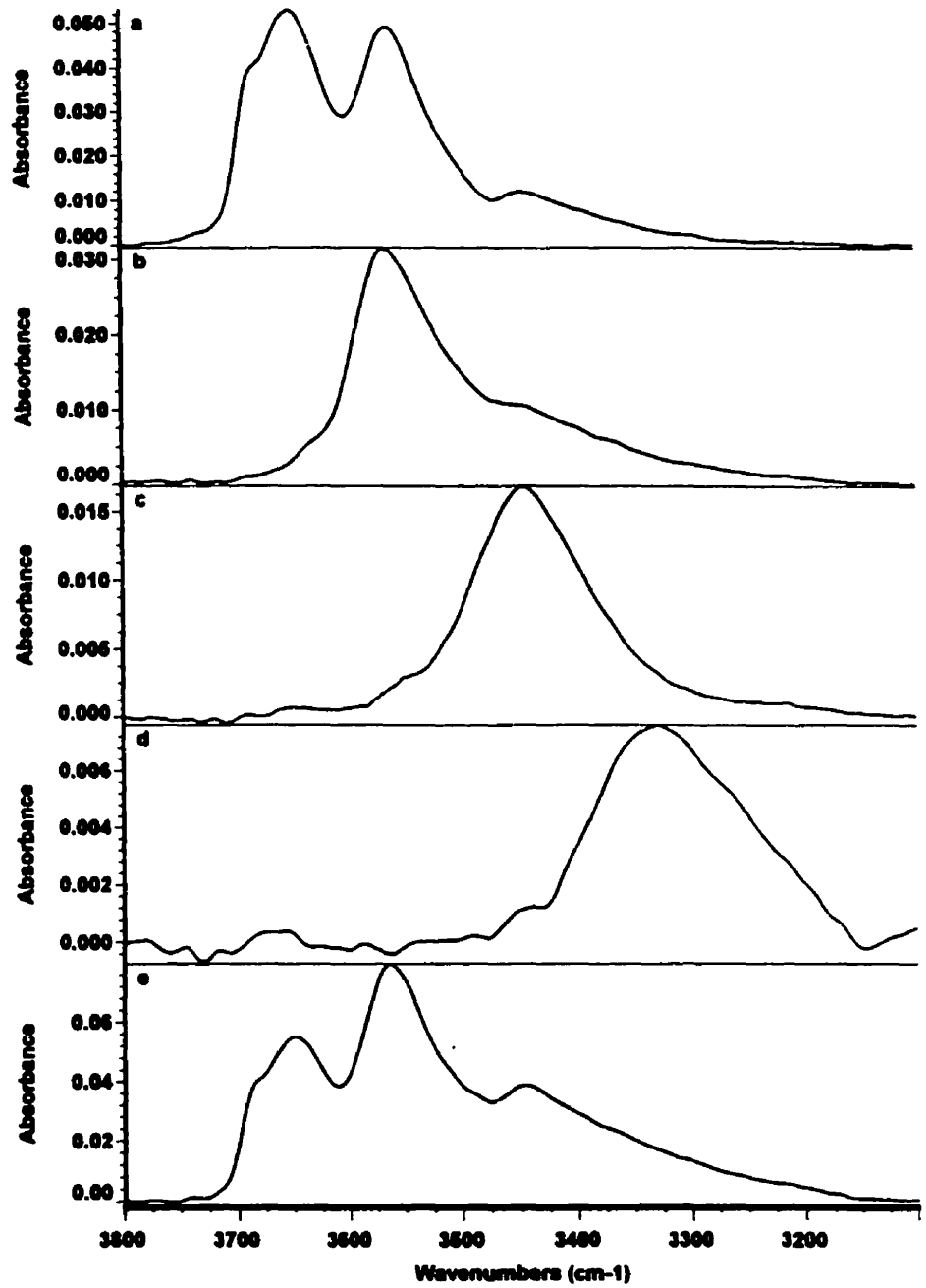


Figure 3.2. The OH stretching region (3800-3100 cm^{-1}) in the ATR/FTIR spectra of oxidation reference compounds obtained by ratioing the single-beam spectra of olive oil spiked with the reference compound against the single-beam spectrum of pure olive oil: (a) H_2O; (b) hexanol; (c) *t*-butyl hydroperoxide; (d) oleic acid; (e) spectrum obtained by co-adding spectra a-d. All reference compounds were added at 2% (w/w), except for H_2O, which was added to saturation levels.

the higher-frequency component of the water band and/or the other characteristic absorption of water at 1625 cm^{-1} (not shown).

Figure 3.3 illustrates the carbonyl region in the hexanal, hexenal, decadienal, hexenone, and oleic acid reference spectra, plus their composite spectrum. The aldehydes all exhibit C=O stretching bands in the 1730-1680 cm^{-1} region, as shown in Figure 3.3a-c, as well as weaker CH stretching bands between 2820 and 2700 cm^{-1} (not shown). The presence of α,β unsaturation causes a substantial shift of the C=O stretching band to lower wavenumbers (from 1727 cm^{-1} for hexanal to 1697 cm^{-1} for *trans*-2-hexenal); this shift is accentuated by the presence of an additional conjugated double bond (1689 cm^{-1} for *trans,trans*-2,4-decadienal). The α,β-unsaturated aldehydes can also be distinguished by their C=C stretching absorption at ~1640 cm^{-1}, which exhibits exceptional intensity due to conjugation with the aldehyde group, and their bands in the *trans*-C=C–H bending region. In the latter region (not shown), *trans,trans*-2,4 decadienal exhibits an additional characteristic absorption at 987 cm^{-1} due to the conjugated *trans* double bonds, whereas *trans*-2-hexenal exhibits a band at 974 cm^{-1}, characteristic of the isolated *trans* double bond. On the basis of the characteristic spectral features of these reference aldehyde compounds, it should be possible to distinguish in general between saturated, α,β-unsaturated, and α,β,δ,γ-unsaturated aldehydes.

Figure 3.3d illustrates the spectrum of *trans*-4-hexen-3-one, an allylic ketone. The C=O stretching absorption is a doublet with one component, corresponding to the *s-cis* isomer (19), appearing where saturated ketones normally absorb, while the second absorption, corresponding to the *s-trans* isomer, is in close proximity to the C=O

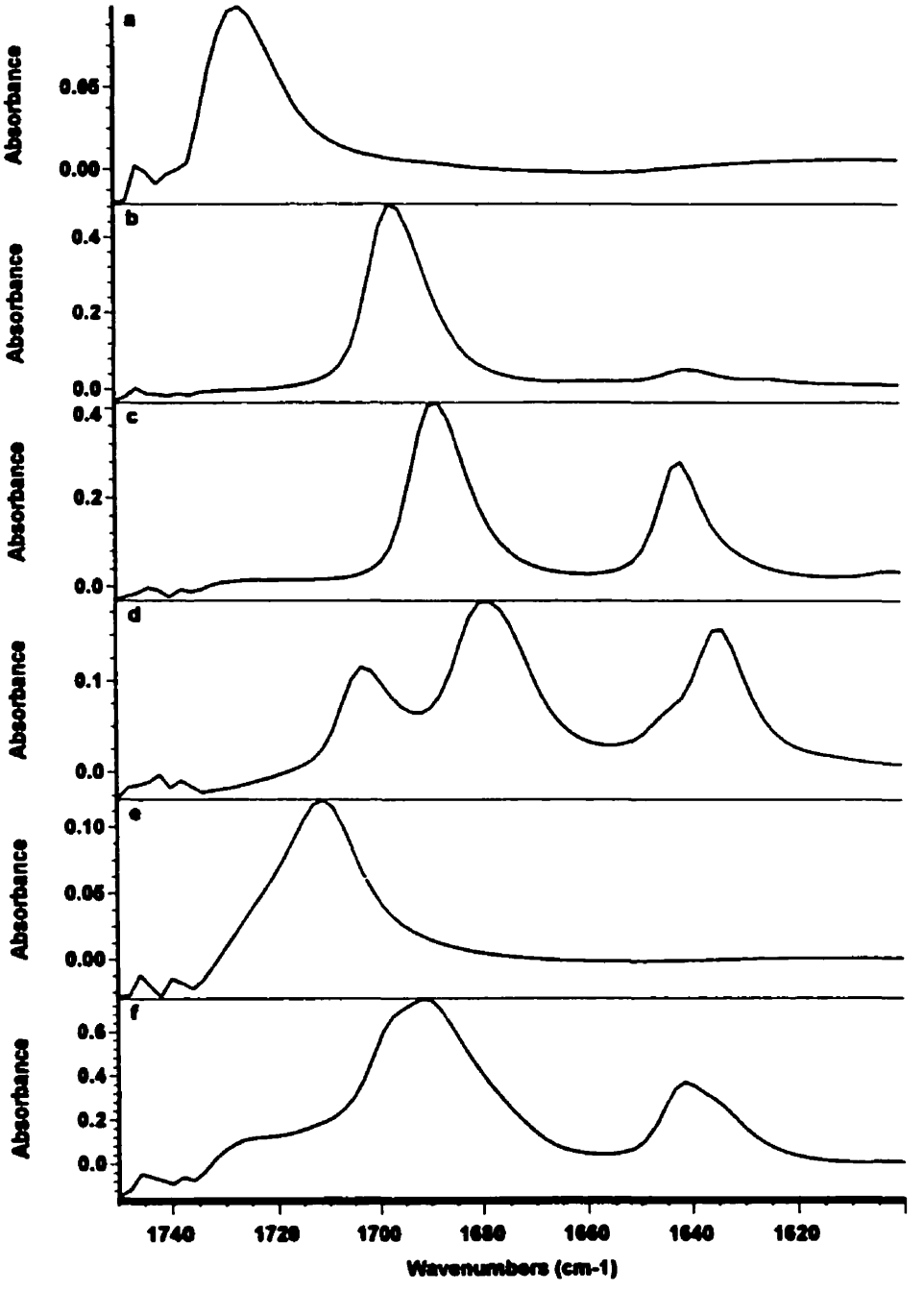


Figure 3.3. The carbonyl region (1750-1600 cm^{-1}) in the ATR/FTIR spectra of oxidation reference compounds obtained by ratioing the single-beam spectra of olive oil spiked with the reference compound against the single-beam spectrum of pure olive oil: (a) hexanal; (b) *trans*-2-hexenal; (c) *trans,trans*-2,4-decadienal; (d) *trans*-4-hexen-3-one; (e) oleic acid; (f) spectrum obtained by co-adding spectra a-e. All reference compounds were added at 2% (w/w).

stretching absorption of *trans,trans*-2,4-decadienal. It also exhibits C=C stretching and *trans*-C=C–H bending absorptions that overlap with the corresponding bands of *trans*-2-hexenal. Thus, it would be difficult to distinguish either saturated or unsaturated ketones from α,β-unsaturated aldehydes in an oxidized oil, especially as these compounds are generally present in trace amounts. Aldehydes, however, can be definitively distinguished from ketones on the basis of their characteristic CH stretching absorptions in the 2820-2700 cm^{-1} region.

The last major functional group of interest is the carboxyl group of FFAs (FFAs), represented by oleic acid, which absorbs at 1711 cm^{-1} (Figure 3.3e), as well as in the OH stretching region, as discussed earlier. The carboxyl group produces a clean, single band in the C=O stretching region; however, as shown in the co-added spectrum (Figure 3.3f), this absorption lies between two aldehyde bands (hexanal and *trans*-2-hexenal) and would be difficult to distinguish in the spectrum of an oxidized oil unless FFAs are present in relatively large amounts.

Overall, based on the co-added spectra in the hydroxyl, carbonyl, and *trans* double bond regions (not shown), the indications are that substantial spectral information should be available if products of the types represented by the reference compounds are formed during the oxidative process. To ascertain whether this expectation is met in real systems undergoing oxidation, a variety of oils were oxidized under accelerated conditions and followed spectrally as a function of time. Examples of the results from these experiments are presented below to illustrate the types of spectral changes observed in oils undergoing oxidation.

3.4.3. Moderate Heating—Safflower Oil

To simulate moderate but accelerated oxidative conditions, safflower oil was placed on a heated (75°C) horizontal ATR accessory. The combination of temperature and the large surface area of the oil that is exposed to air speeds up the autoxidative process. As such, the system simulates the active oxygen method (AOM), except that oxidative changes can be monitored in real time as oxidation proceeds. Figure 3.4 illustrates the spectra recorded at t = 1, 8, and 16 h, ratioed against the spectrum recorded at t = 0. These spectra were acquired on a 45° ZnSe crystal to minimize digitization noise in regions of intense oil absorptions. As oxidation progresses with time, one can readily see, from left to right in Figure 3.4: (a) the appearance of a broad band in the OH stretching region (3800-3200 cm^{-1}), (b) a decrease in the CH stretching vibrations (3050-2800 cm^{-1}), (c) a large decrease in the triglyceride ester linkage absorption (1744 cm^{-1}), (d) the appearance of peaks on the low-frequency side of the triglyceride ester linkage absorption, and (e) a sharp rise in peaks in the *trans* double bond region (1000-900 cm^{-1}). All positive bands in Figure 3.4 are an unambiguous indication of the production of new molecular species. However, negative bands do not necessarily represent the loss of functional groups. For example, the sharp decrease in the triglyceride ester linkage absorption could be taken to imply that an extensive amount of hydrolysis has occurred; however, not only is this unlikely under these moderate conditions, it definitely does not occur, as there is no spectral evidence of concurrent FFA formation. In fact, the decrease in the ester linkage absorption (and in some of the CH absorptions) is caused by oxygen being chemically incorporated into the oil as oxidation products are formed. This oxygen uptake, in effect, dilutes the sample relative to the reference, and, as a consequence, bands

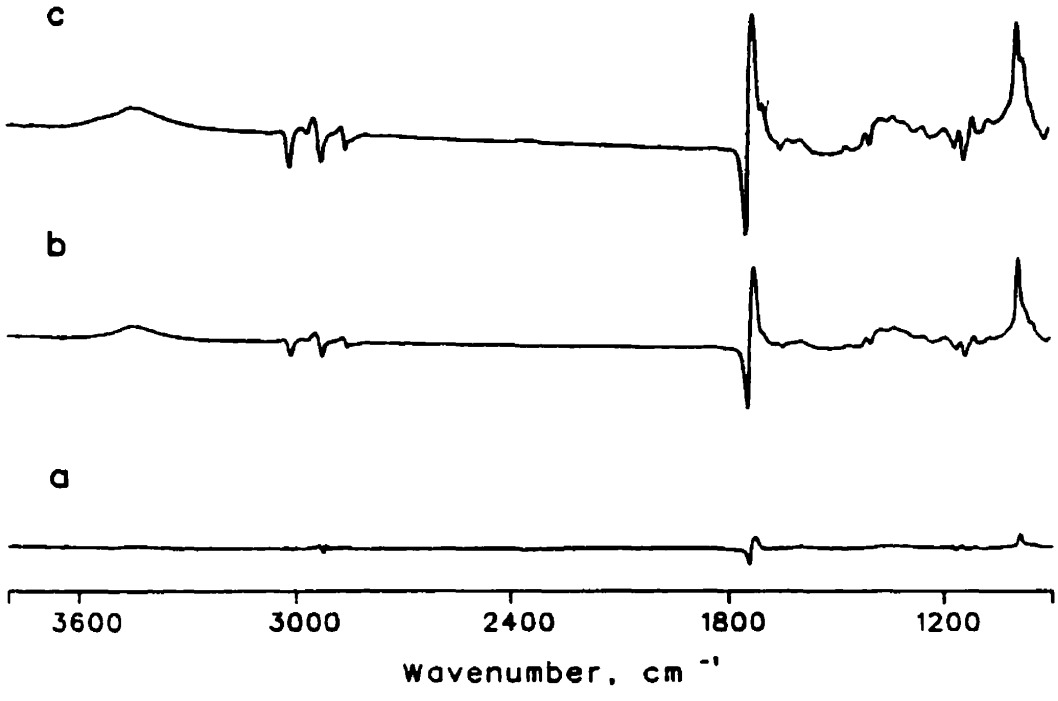


Figure 3.4. ATR/FTIR spectra of safflower oil heated to 76°C on the surface of a 45° ZnSe ATR crystal: (a) t = 1 h; (b) t = 8 h; (c) t = 16 h. The spectra have been ratioed against the spectrum recorded at t = 0.

that are common to the sample and the reference oil do not ratio out perfectly, but instead exhibit negative intensity in the ratioed spectrum. For each absorption band of the oil, the negative band intensity in the ratioed spectrum due to this oxygen uptake effect is proportional to its intensity in the oil's absorbance spectrum (i.e., its spectrum ratioed against air), and thus the greatest effects are observed for the strongest absorption bands, such as the triglyceride ester linkage absorption. One can determine whether a negative band is due solely to the oxygen uptake effect or to a combination of this effect and the actual loss of a functional group by comparing its intensity to that of a strong reference band associated with a stable and relatively unreactive functional group. The absorption band of the triglyceride ester linkage, which is one of the more stable bonds in an oil system unless thermally stressed (>200°C), can serve as such a reference band provided that the pathlength of the IR cell is sufficiently narrow to avoid digitization noise on ratioing this band (e.g., 45° ZnSe ATR). Under these conditions, dividing the peak height of the (negative) ester linkage band in the ratioed spectrum obtained at t = x h by the height of this band in the absorbance spectrum (i.e., ratioed against air; Figure 3.1a) recorded at t = 0 provides a measure of the dilution of the sample due to the oxygen uptake effect:

$$\begin{aligned}\%\ \text{dilution}\ (t = x\ h) &= \%\ \text{decrease in peak height at } 1744\ cm^{-1} \\ &= |A_{1744}^{r}(t = x\ h)|/A_{1744(t=0)} \times 100 \qquad [3.2]\end{aligned}$$

where A_{1744}^{r} and A_{1744} are the peak heights of the triglyceride ester linkage absorption in the ratioed spectrum at t = x h and the absorbance spectrum at t = 0, respectively. As the extent of dilution is proportional to the amount of oxygen taken up by the sample, this

measure is, in effect, an indicator of the overall extent of oxidation. The percent decrease in peak height that any other negative band represents can be calculated in the same manner [$|A_\nu^r(t = x\ h)|/A_\nu(t = 0) \times 100$] and compared to the value obtained for the ester linkage band (see inset in Figure 3.5). A definitive difference in these values is indicative that changes beyond the oxygen uptake effect contribute to the negative band intensity. With these considerations in mind, it is useful to examine each of the major spectral regions in some detail.

(a) OH Region: Figure 3.5 shows a detailed view of the 3800-2800 cm^{-1} region for the same experiment as in Figure 3.4. The broad peak centered at 3444 cm^{-1} is the O-H stretching vibration of hydroperoxides, a peak assignment established by early work on the oxidation of methyl linoleate (15). There is no indication of alcohol being formed in that the peak remains symmetrical throughout the experiment, as judged by measuring the intensities at points 100 cm^{-1} on either side of the maximum, nor is there any evidence of water or FFA formation. The progressive increase in the hydroperoxide band with time is accompanied by a decrease in the peak at 3011 cm^{-1}, assigned to the CH stretching vibration of *cis* double bonds. This represents a net loss of *cis* double bonds, as opposed to an oxygen uptake effect, as seen by the much greater percent decrease in this band relative to the triglyceride ester linkage band (Figure 3.5, inset). The negative band at 2922 cm^{-1} (Figure 3.5), assigned to the CH_2 stretching vibration, although comparable in magnitude to the *cis* band, can be largely attributed to an oxygen uptake effect, given that this band is an order of magnitude stronger than the *cis* band in the absorbance spectrum (see Figure 3.1a). Hence, Figure 3.5 clearly indicates that there is significant

formation of hydroperoxides coupled with a concomitant loss of *cis* double bonds, a well-known characteristic of lipids undergoing oxidation under moderate conditions (2).

(b) Carbonyl Region: Figure 3.6 illustrates the changes that take place in the carbonyl region (1750-1650 cm^{-1}), excluding the triglyceride ester linkage band discussed earlier. The most significant change is the appearance of two bands at 1726 and 1698 cm^{-1}, with these positions being almost identical to those associated with the characteristic absorptions seen for hexanal and 2-hexenal in olive oil, respectively (Figure 3.3a and b), and it is likely that these peaks are representative of saturated and α,β-unsaturated aldehydes. There is no indication in these spectra that any FFAs are formed under these conditions, as there is no overt absorption band at 1711 cm^{-1}. As noted in our discussion of the library reference spectra, the identification of ketones in the presence of aldehydes is difficult, so it is not possible to determine from Figure 3.6 whether ketones are being produced. Within the region shown in Figure 3.6, there is a small negative band centered at 1650 cm^{-1}, which is due to the C=C stretching absorption of unsaturated fatty acids in the triglycerides. A plot of the type presented in Figure 3.5 showed that the negative intensity of this band is not solely due to an oxygen uptake effect but can be attributed to a net loss of C=C bonds. However, changes in the C=C stretching absorption in the spectra of oxidized oils would generally be difficult to interpret because of the shifts in position and large differences in the extinction coefficient of this absorption for *cis* and *trans* double bonds and for double bonds conjugated with carbonyl groups, such as are commonly found in products of oxidative breakdown of oils.

(c) trans *Double Bond Region*: Major changes take place, as oxidation proceeds, in the region between 1000 and 900 cm^{-1} (Figure 3.7), where the C=C–H bending

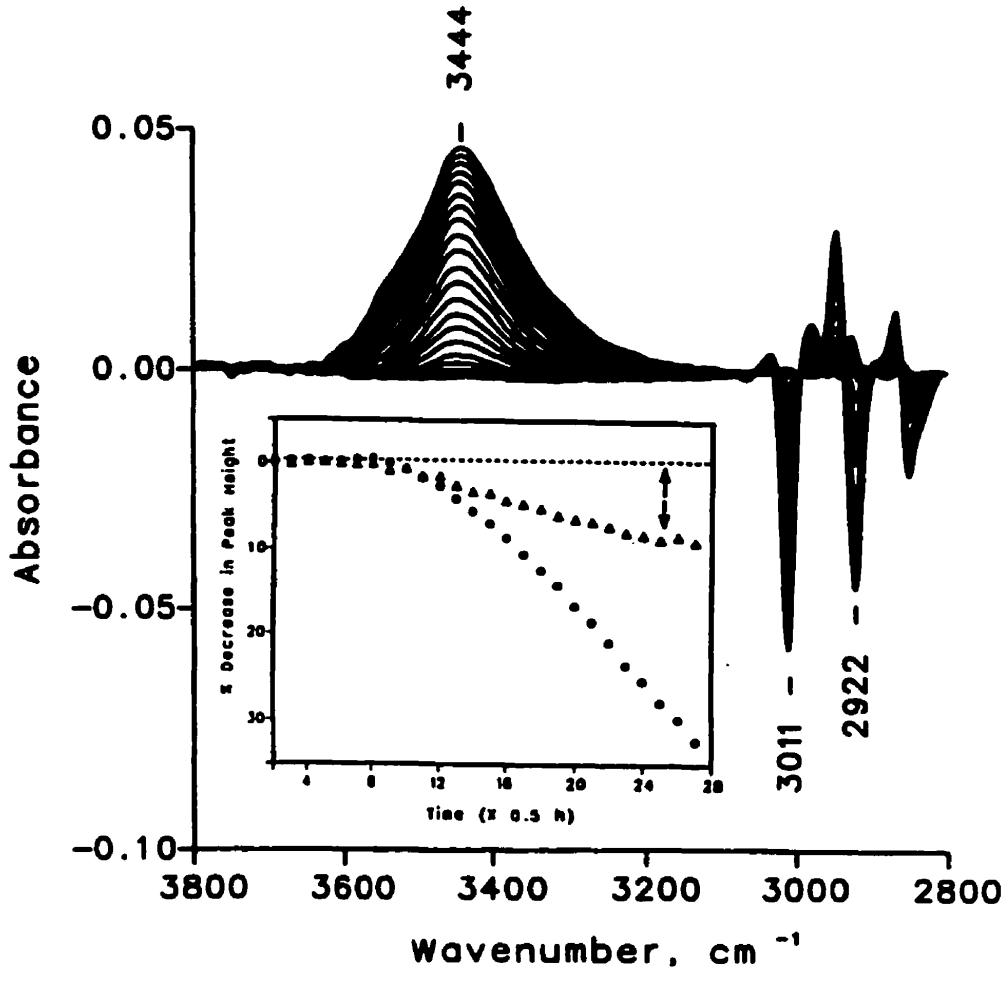


Figure 3.5. Spectral overlay plot illustrating changes in the 3800-2800 cm^{-1} region of the ATR/FTIR spectrum of safflower oil heated to 76°C on the surface of a 45° ZnSe ATR crystal as a function of time. The overlaid spectra were recorded at 30-min intervals and have been ratioed against the spectrum recorded at t = 0. *Inset:* Percent decrease in the peak heights of the *cis*-C=CH stretching (•) and triglyceride ester linkage (▲) bands vs. time. The arrow indicates the magnitude of the dilution of the oil due to the oxygen uptake effect.

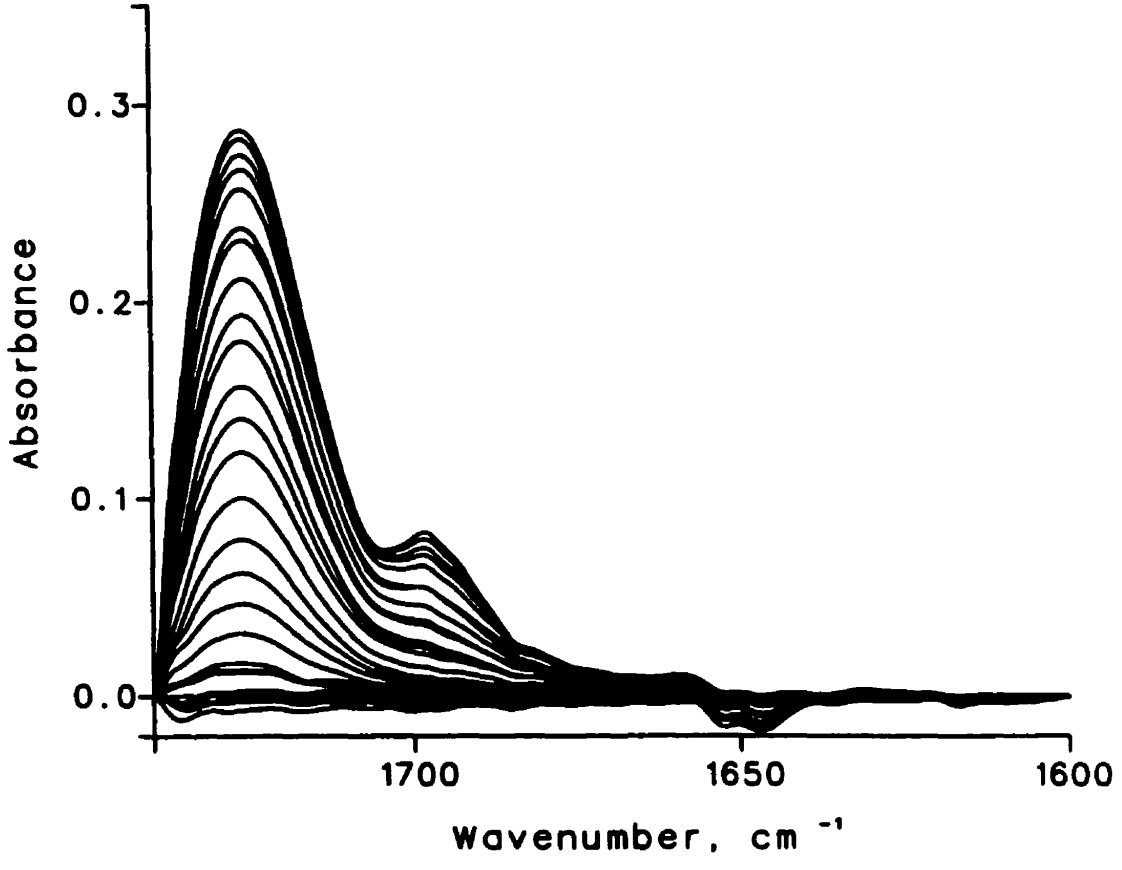


Figure 3.6. Spectral overlay plot illustrating changes in the 1750-1600 cm^{-1} region of the ATR/FTIR spectrum of safflower oil heated to 76°C on the surface of a ZnSe ATR crystal as a function of time. The overlaid spectra were recorded at 30-min intervals and have been ratioed against the spectrum recorded at t = 0.

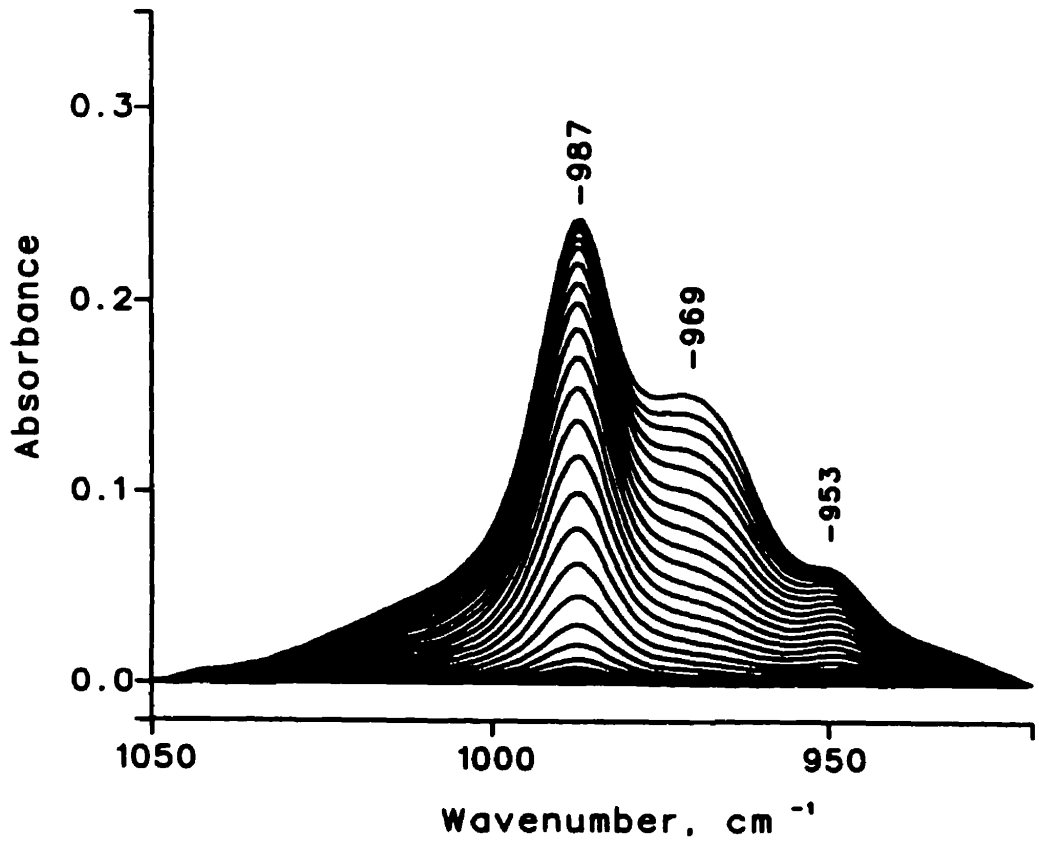


Figure 3.7. Spectral overlay plot illustrating changes in the 1050-900 cm^{-1} region of the ATR/FTIR spectrum of safflower oil heated to 76°C on the surface of a ZnSe ATR crystal as a function of time. The overlaid spectra were recorded at 30-min intervals and have been ratioed against the spectrum recorded at t = 0.

vibrations of *trans* double bonds occur. The appearance of two bands at 987 and 953 cm^{-1}, in approximately a ~2:1 ratio, is apparent in the early stages of oxidation, while, later on, the band at 987 cm^{-1} exhibits a slight shift toward higher wavenumbers and a shoulder on this band, at 969 cm^{-1}, becomes apparent. This region has been extensively investigated in the spectra of hydroperoxides formed and isolated from methyl linoleate undergoing oxidation (16), with the peaks observed at 982 and 948 cm^{-1} (in CS_2 solution) assigned to *cis,trans* conjugated dienes. In the spectrum of a mixture of *trans,trans* and *cis,trans* conjugated dienes, a shift of the 982 cm^{-1} band to 985 cm^{-1} was observed, whereas the pure *trans,trans* conjugated diene was shown to exhibit a single band at 988 cm^{-1}. Taking into account the slight shifts in peak positions on going from CS_2 solution to neat oil, Figure 3.7 demonstrates the formation of conjugated dienes, with the amount of *trans,trans* conjugated dienes relative to *cis,trans* conjugated dienes increasing as a function of time, as well as the formation of isolated *trans* bonds, indicated by their characteristic absorption at 969 cm^{-1}.

3.4.4. Real-Time Oxidation Plots

Based on the results shown in Figures 3.4-3.7, it is possible, by using the FTIR ratio method, to follow the relative changes in the appearance or disappearance of the major functional groups in an oil as a function of time as oxidation proceeds. To obtain a graphical representation of the relative changes in the functional group absorptions during the course of oxidation, the spectrometer was programmed to measure the absorbance values (peak heights) at the wavenumbers listed in Table 7.1 for the peak maxima of the functional group absorptions at specified time intervals during oil oxidation experiments. The values at each wavelength measured were subsequently normalized with respect to

the maximum absorbance value reached at that wavelength ($A_\lambda/A_\lambda^{max}$) during the course of the experiment and plotted against time to produce a plot that represents the course of the oxidation process (real-time oxidation plot).

(a) Oxidation at Moderate Temperatures—Safflower/Cottonseed Oil. Figure 3.8 is a real-time oxidation plot for the initial stages (15 h at 76°C) of autoxidation of safflower oil, showing the relative changes in the hydroperoxide, aldehyde, *cis*, and *trans* absorbances as a function of time. As such, it clearly shows that after an initial period of stability (induction period), there is a loss of *cis* double bonds with a concomitant appearance of hydroperoxides, aldehydes, and *trans* double bonds. No alcohols or FFAs were in evidence in this experiment. In contrast, in a subsequent experiment on cottonseed oil, oxidized at the same temperature but for a longer period of time (50 h), a more complicated real-time oxidation plot is obtained, which has been separated into three frames for clarity (Figure 3.9a-c). Once again, there is a rapid depletion of *cis* double bonds and the production of aldehydes (Fig 3.9a), with α,β-unsaturated aldehydes being the last to appear. Figure 3.9b illustrates the *trans,trans* and *cis,trans* conjugated dienes rising steadily, reaching a maximum, and subsequently beginning to decrease, while the band due to isolated *trans* bonds rises rapidly and then begins to plateau. Figure 3.9c illustrates that both hydroperoxides and alcohols are being formed in this system, showing an initial rise up to 20 h, followed by a drop and plateau, respectively, subsequently followed by a rise in the relative absorbance values of both products. A closer examination of this region of the spectrum (Figure 3.9d) indicates that, in actual fact, hydroperoxides (▲) are produced initially, maximize, and then start to decrease;

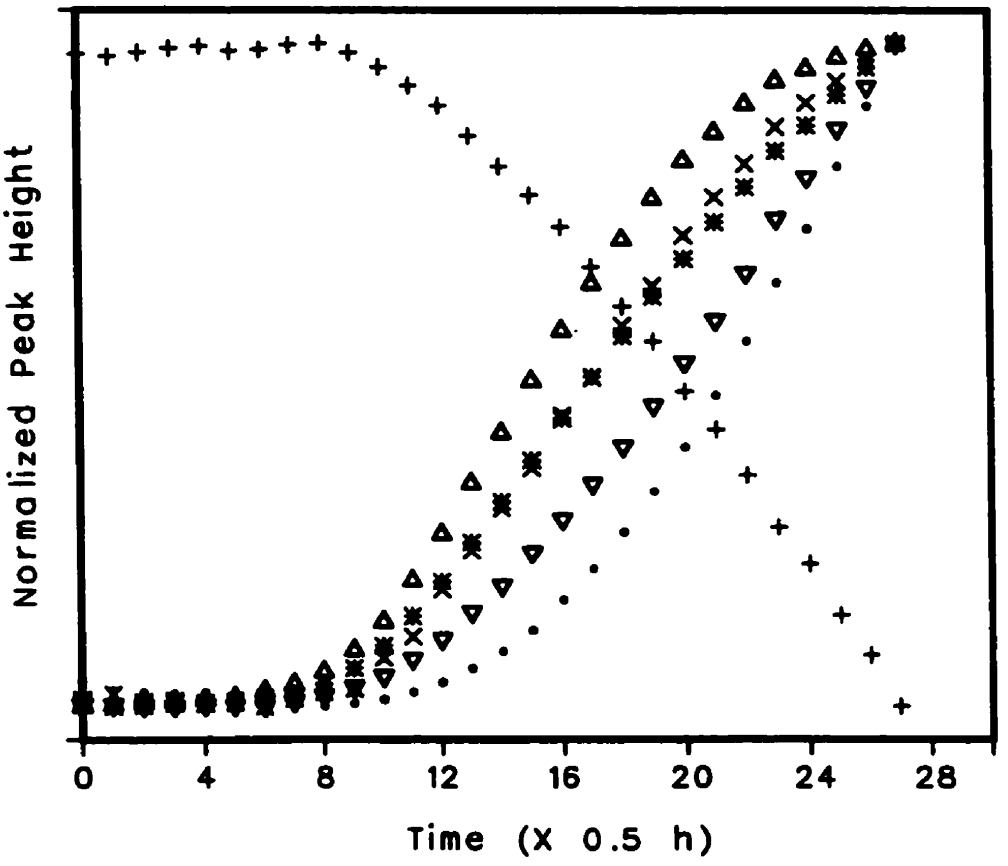
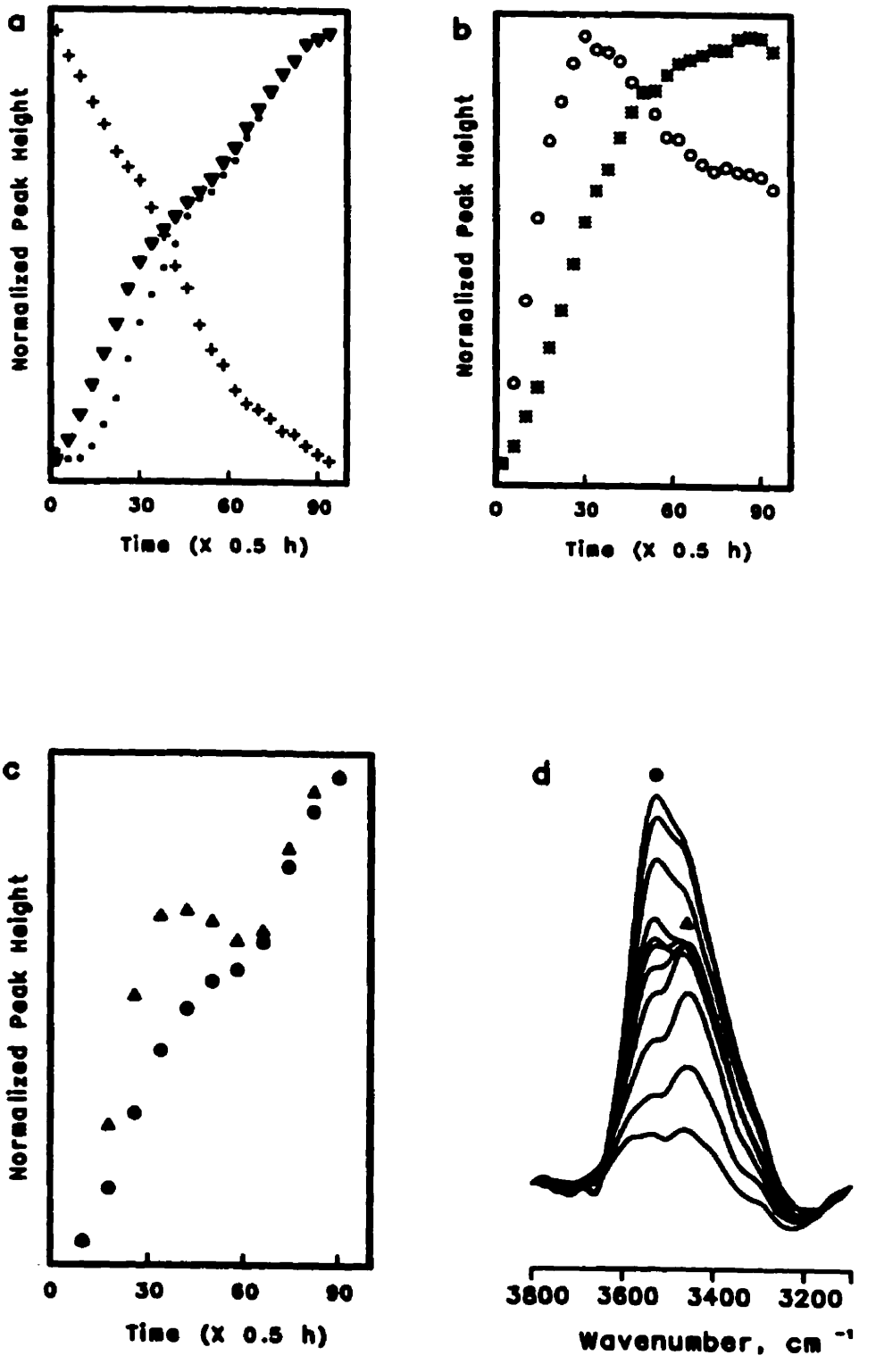


Figure 3.8. Real-time oxidation plot for safflower oil at 76°C, showing the normalized peak heights of the major functional group absorption bands vs. heating time: +, *cis* C=C–H stretching absorption (3011 cm^{-1}); Δ, *trans* C=C–H conjugated (987 cm^{-1}); ×, ROOH (3444 cm^{-1}); ∗, $R'CH_2CHO$ (1726 cm^{-1}); ∇, *trans* C=C–H isolated (969 cm^{-1}); ∘, R"C=C–CHO (1698 cm^{-1}).

Figure 3.9. Partitioned real-time oxidation plot for cottonseed oil at 76°C: (a) +, *cis* C=C–H stretching absorption (3011 cm^{-1}); ∇, $R'CH_2CHO$ (1726 cm^{-1}); •, R"C=C–CHO (1698 cm^{-1}); (b) ○, *trans* C=C–H conjugated (987 cm^{-1}); *, *trans* C=C–H isolated (969 cm^{-1}); (c) ▲, ROOH (3444 cm^{-1}); ●, R'OH (3544 cm^{-1}); (d) overlaid spectra in the OH stretching region, illustrating the overlap of the ROOH and R'OH bands.


a
Normalized Peak Height
0
30
60
90
Time (X 0.5 h)
b
Normalized Peak Height
0
30
60
90
Time (X 0.5 h)
c
Normalized Peak Height
0
30
60
90
Time (X 0.5 h)
d
3800
3600
3400
3200
Wavenumber, cm -1

however, the absorbance values measured for the hydroperoxide band continue to rise due to overlap with the continually rising alcohol band (●). The inflection point at ~30 h in the alcohol plot is also an anomaly due to the alcohol band being pulled down by the decrease in the overlapping hydroperoxide band. Referring back to Figure 3.9b, a similar effect causes the plateau in the isolated *trans* plot, the band being dragged down by the concurrent drop in the *cis,trans/trans,trans* double bond signal, due to the close proximity of these two bands. When spectral subtractions were carried out to compensate for these overlaps, it was found that both the hydroperoxide and the conjugated diene absorptions had, in fact, dropped to negligible values by the end of the experiment. Thus, although real-time oxidation plots provide an excellent overview of the chemical changes taking place as oils oxidize, some caution is required in interpreting them in absolute terms, especially in the case of hydroperoxides.

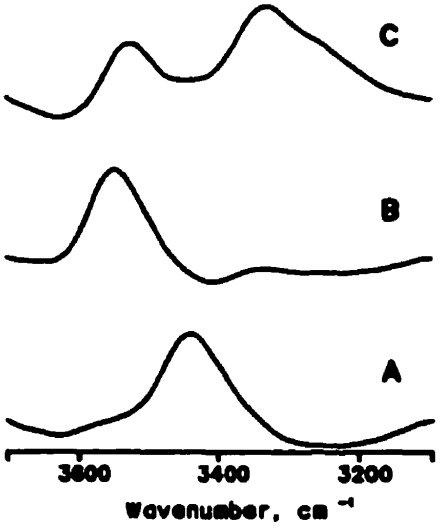
(b) Thermally Stressed Oil—Safflower. To study the oxidation behavior of oils under various degrees of thermal stress, FTIR spectra were recorded in the transmission mode, the oil being heated and oxidized in a reservoir and circulated continuously through a transmission flow cell. Safflower oil was heated to 100, 130, and 230°C for 40, 160, and 200 min, respectively, with spectra recorded every 20 min. Figure 3.10 illustrates spectra recorded at the end of each heating stage over the three main spectral regions. Looking at the OH stretching region, only hydroperoxides are present after 40 min at 100°C, while after 160 min at 130°C, the hydroperoxides have disappeared and alcohols have formed. Under extreme thermal stress (230°C for 200 min), there is an extensive formation of FFAs, as indicated by the appearance of a band at 3324 cm^{-1} (OH

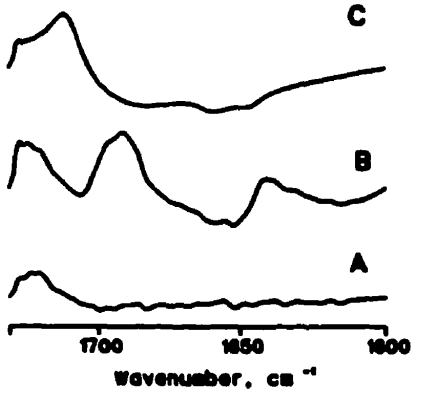
stretching band of the carboxyl group), as well as alcohols; however, the alcohol band appears to have shifted slightly. In the carbonyl region, at 100°C, a small amount of unsaturated aldehydes is evident, while at 130°C α,β-unsaturated aldehydes have formed in significant amounts, and saturated aldehydes have increased further. At 230°C, the predominant band in this region is at 1711 cm^{-1} due to the formation of FFAs, with some saturated aldehydes remaining, but α,β-unsaturated aldehydes have disappeared completely. The spectra in the *trans* double bond region illustrate the initial formation of *cis,trans* and *trans,trans* conjugated dienes at 100°C, followed by the progressive conversion of the conjugated bonds to isolated *trans* bonds on going to 130 and 230°C.

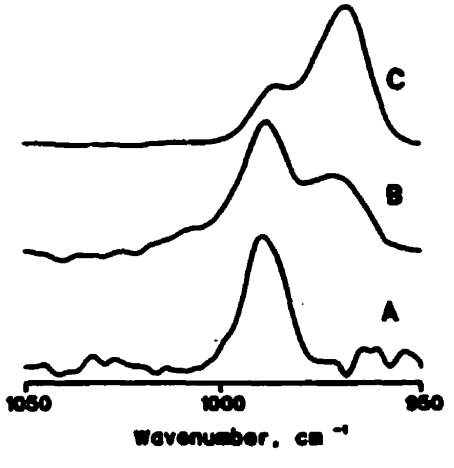
A real-time oxidation plot is presented for only the alcohols and the FFAs in Figure 3.11a. It can be seen that raising the temperature to 230°C initiates thermal hydrolysis, as evidenced by the rapid increase in FFAs. There is a concurrent slope change in the alcohol plot just as the FFAs begin to be released. Figure 3.11b illustrates the detailed spectral changes associated with the real-time oxidation plot (Figure 3.11a). There is a band shift in the alcohol region coinciding with the appearance of FFAs. Interpreting these spectra, there is a shift from the production of alcohols from hydroperoxide breakdown (3544 cm^{-1}) to the appearance of alcohol functional groups associated with mono- and diglycerides (3526 cm^{-1}), produced when FFAs are liberated. Although this is a minor spectral change, it demonstrates that one can even differentiate between different types of alcohols in the spectra of oxidized oils.

Although the oxidation conditions and oils studied here are not all-inclusive, they were adequate to produce most of the compounds commonly associated with lipid

Figure 3.10. The FTIR spectra in the OH stretching (3650-3200 cm^{-1}), carbonyl (1750-1600 cm^{-1}), and *trans* double bond regions (1050-950 cm^{-1}) of thermally stressed safflower oil, obtained by ratioing the single-beam spectrum recorded after 40 min at 100°C (A), 160 min at 130°C (B), and 200 min at 230°C (C) against that recorded at the beginning of the experiment.


C
B
A
3600
3400
3200
Wavenumber, cm -1


C
B
A
1700
1650
1600
Wavenumber, cm -1


C
B
A
1050
1000
950
Wavenumber, cm -1

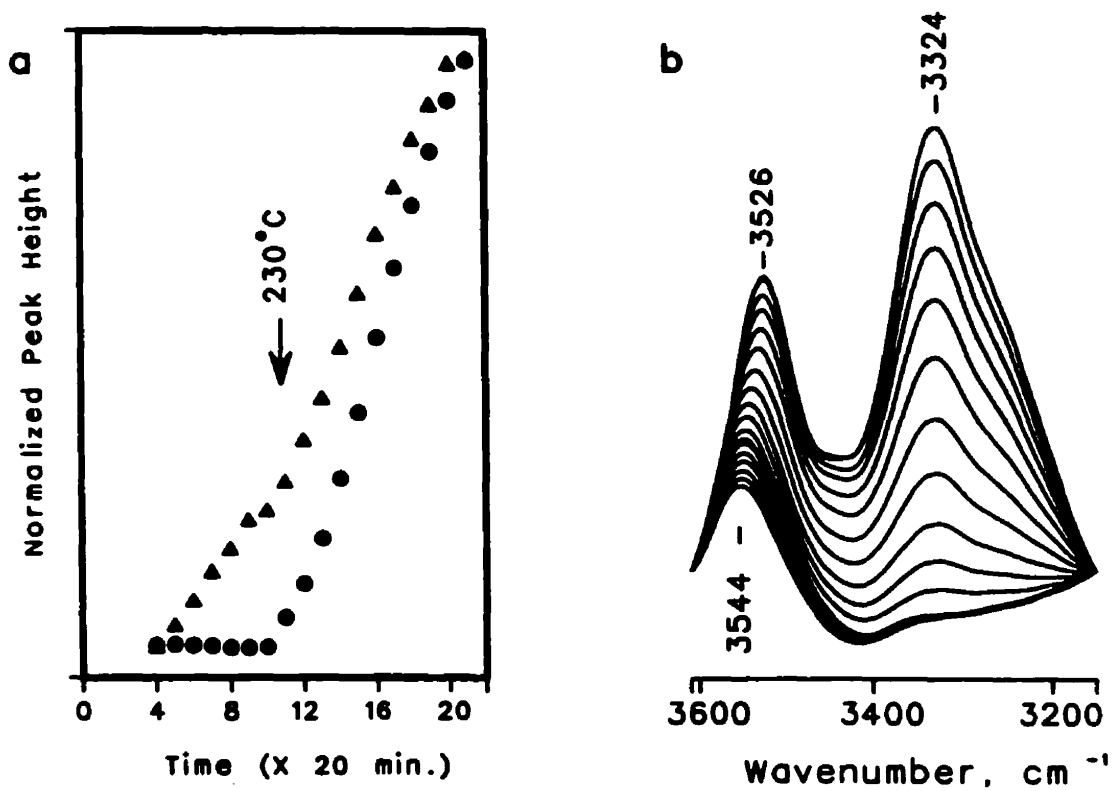


Figure 3.11. (a) Partial real-time oxidation plot for the RO–H (▲, 3544 cm^{-1}) and RC(=O)O–H (•, 3324 cm^{-1}) stretching absorptions for safflower oil heated from 130°C to 230°C and then maintained at 230°C for 200 min; (b) corresponding overlaid spectra, illustrating the shift in the OH stretching absorption maximum from 3544 to 3526 cm^{-1} as the temperature is increased.

oxidation and serve to illustrate the information obtainable by FTIR spectroscopy in monitoring oil oxidation. Practical applications based on this approach can be envisaged, e.g., real-time monitoring of oil on a heated ATR crystal being utilized as a modified AOM or use of a flow-cell system for on-line monitoring of frying oils.

3.4.5. Quantitative Aspects

Based on the results obtained from this limited spectroscopic survey of changes taking place in oils undergoing oxidation, it is clear that relative changes can be readily measured, although there are some problems in interpreting such changes in the case of overlapping bands (e.g., alcohol and hydroperoxide absorptions). Generally, the key products of interest in assessing the oxidative status of oils are hydroperoxides, alcohols, and aldehydes, with FFAs becoming important in high-temperature-stressed oils. Judging from our spectral study, FTIR spectroscopy could be further developed into a relatively simple technique for the quantitative determination of common oxidation products in oils. We have already developed a quantitative method for the determination of FFAs in oils based on the ratio concept presented in this paper (13); however, a number of considerations require elaboration for the further development of a generalized method of quantitation, as outlined below.

(a) Obtaining a Reference Oil. A crucial consideration in making use of the ratio method is that one should have a high degree of confidence that the reference oil is free of oxidative contaminants. If a compound to be analyzed for, or some other contaminant absorbing at the frequency used for its measurement, is present in the reference oil, the absorption band of this compound in the ratioed spectrum of the sample will be reduced in intensity to the extent that the contaminant is present in the reference oil. As such, one

would detect changes relative to the reference oil but would not obtain an accurate concentration value. This is not a problem if one is confident that the reference oil is "fresh"; however, it is best to ensure a zero baseline by cleaning up the reference oil. A simple procedure is to pass the oil through activated silica gel, which removes all compounds with any degree of polarity (water, alcohols, hydroperoxides, aldehydes, FFAs, etc.). The efficacy of this procedure was assessed spectrally and confirmed by PV, AV, and FFA determinations. To determine spectroscopically that alcohols, FFAs, and hydroperoxides were completely removed, the spectrum of the purified oil was ratioed against that of a fraction of this oil that had been treated with D_2O. This treatment converts all OH functional groups to OD functional groups, which absorb at a lower frequency owing to their higher mass, and thus ratioing against the spectrum of the D_2O-treated sample provides a clear window in the OH stretching region. No OH bands were observed in the spectra of the silica gel-treated samples, indicating that alcohols, FFAs, and hydroperoxides were completely removed. Hence, the silica gel treatment provides a simple means of obtaining a "clean" reference oil.

(b) Oxidation Calibration Standards. With the availability of a "clean" reference oil, it is possible to prepare calibration standards by spiking this oil with compounds that are spectroscopically representative of the major oxidation products, e.g., *t*-butyl hydroperoxide, hexanol, hexanal, *trans*-2-hexenal, and *trans,trans*-2,4-decadienal. This approach is both simple and practical and expresses the absorbance values of oil samples in terms of the equimolar concentrations of the reference compounds. In formulating a quantitative approach of this nature, one has to consider the potential interferences due to the overlap of water, hydroperoxide, and alcohol absorptions in the OH region and the

extensive overlap of bands in the carbonyl region. These interferences can be accounted for by preparing a set of standards containing randomized quantities of the reference compounds in a clean oil and carrying out multivariate analysis, which provides cross corrections for the overlapping bands. A number of mathematical approaches are available, the simplest being multiple regression based on peak height measurements. Alternatively, FTIR chemometric packages, based on **P**- or **K**-matrix (20, 21) or partial-least-squares (PLS) methods (22), may be employed. Such software works directly with spectral data and can typically abstract absorbance values and/or integrated intensities from the spectra, calculate the calibration factors, and print out the concentrations of unknowns. Once a calibration is achieved, it would be useful to express the oxidative state of an oil in terms of % *t*-butyl hydroperoxide, % hexanol, and, by summing all the aldehyde and ketone contributions, % total carbonyls. Alternatively, similar techniques could be used to relate selected absorptions to well-known chemical measures of oxidation, e.g., PV and AV. Either approach would take some effort to develop; however, once this is done, the spectroscopic and mathematical elements could be preprogrammed, by using the macro-programming capabilities of the FTIR software, resulting in a method that would be simple and directly applicable to the quality control of oils, but requiring no spectroscopic knowledge to carry out an analysis.

3.5. CONCLUSION

This study demonstrates the power and utility of FTIR spectroscopy in following and determining the relative state of oxidation of an oil under a variety of conditions. The capability to assess the relative oxidative state of an oil sample or to monitor oil oxidative

processes by FTIR spectroscopy in real time should be especially useful for the general quality control of edible oils. The next challenge will be to develop, program, and assess the quantitative approaches suggested and establish whether correlations between FTIR spectral changes and conventional chemical methods can be obtained, so that the FTIR results can be expressed in analytical terms with which the edible oil industry is familiar.

ACKNOWLEDGMENTS

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BRIDGE

The objective of the research described in Chapter 3 was to investigate the potential application of FTIR spectroscopy in the assessment of the oxidative status and stability of edible oils. The results obtained in this study laid the foundation for subsequent work by the McGill IR Group that led to the development of quantitative methods for the determination of PV and AV, which were reviewed in Chapter 2. In accordance with the global aim of examining the utility of FTIR spectroscopy as a quality control tool for the fats and oils industry, presented in Chapter 1, the focus of the research described in the following four chapters shifts from the assessment of oxidative status and stability to the bulk characterization of fats and oils.

In the previous chapter, the spectral ratioing capabilities of FTIR spectroscopy were exploited to obtained detailed information on the spectral changes that occurred when oils underwent oxidation as well as to monitor the time course of oxidation. In Chapter 5, the utility of spectral ratioing as a means of eliminating spectral interferences in the determination of the *trans* content of fats and oils from a simple univariate calibration will be demonstrated. First, however, the chemometric tools for quantitative IR analysis that have come to the fore with the advent of the personal computer are shown, in the upcoming chapter, to allow for the simultaneous determination of IV, *cis* and *trans* content, and SN from the FTIR spectrum of an oil.

CHAPTER 4

A RAPID, AUTOMATED METHOD FOR THE DETERMINATION OF *CIS* AND *TRANS* CONTENT OF FATS AND OILS BY FTIR SPECTROSCOPY

4.1. ABSTRACT

A rapid Fourier transform infrared (FTIR) method was developed to simultaneously determine percent *cis* and percent *trans* content of edible fats and oils. A generalized industrial sample-handling platform/accessory was designed for handling both fats and oils and was incorporated into an FTIR spectrometer. The system was calibrated to predict the *cis* and *trans* contents of edible oils by using pure triglycerides as standards and partial-least-squares (PLS) regression as the chemometric approach. The efficacy of the calibration was assessed by triglyceride standard addition, by mixing of oils with varying *cis*/*trans* contents, and by analyzing fats and oils of known iodine value (IV). Each of the approaches verified that the FTIR method measured the *cis* and *trans* contents in a reproducible (±0.7%) manner, with the measured accuracies being 1.5% for standard addition and 2.5% for the chemically analyzed samples. Comparisons were also made to the conventional American Oil Chemists' Society (AOCS) method for the determination of *trans* isomers by IR spectroscopy. The FTIR-PLS approach worked well over a wide range of *trans* contents, including those between 0 and 15%. The sample-handling accessory designed for this application was shown to be robust, flexible, and easy to use, being particularly suited for quality control applications. In addition, the analysis was automated by programming the spectrometer in Visual Basic (Windows), to provide a simple, prompt-based user interface that allows an operator to carry out

cis/trans analyses without any knowledge of FTIR spectroscopy. A typical analysis requires less than two minutes per sample. The derived calibration is transferable between instruments, eliminating the need for recalibration. The integrated analytical system provides a sound basis for the implementation of FTIR methods in place of a variety of AOCS wet chemical methods when analytical speed, cost, and environmental concerns are issues.

4.2. INTRODUCTION

Edible fats and oils are, by definition, either solid or liquid at room temperature, their physical state being defined by their triglyceride makeup, relative degree and form of unsaturation (*cis* or *trans*), weight-average molecular weight or saponification number (SN), and overall fatty acid composition/distribution. Of these complex determinants that define the physical state of a triglyceride lipid system, reducing the degree of unsaturation is the most common means used by industry to convert oils into fats. The majority of the unsaturated fatty acids making up edible oil triglycerides are normally found in the *cis* form. When oils are hardened by hydrogenation for their use in formulating margarines and shortenings or partially hydrogenated to stabilize them to oxidation, there is the concurrent conversion of *cis* to *trans* double bonds. As *trans* fatty acids have a higher melting point, they also contribute to the net hardening effect. However, the presence of substantial amounts of *trans* fatty acids has become controversial due to their association with arteriosclerosis and heart disease (1). The levels of *trans* fatty acids can reach values of 40% or more in hardened fats, and legislation is being considered by the U.S. Food and Drug Administration to require the labeling of the *trans* content of hydrogenated fats and

oils. As such, it is becoming imperative for processors to be able to rapidly determine the *trans* and/or *cis* content of processed fats and oils, so that they can better control the hydrogenation process.

The McGill IR Group has been working on the development of new, rapid, and accurate methods for food and edible oil analysis based on FTIR spectroscopy (2). This technology is a major advance over conventional dispersive-based IR spectroscopy (3) and, being based on interferometry, provides enhanced energy throughput and better signal-to-noise ratio and incorporates substantial computing, chemometric, and macro-programming capabilities. As such, FTIR instruments can be preprogrammed and automated to carry out analyses on a routine basis.

Edible oils are ideal candidates for quality control applications of FTIR spectroscopy. To date, methods have been developed for IV and SN (4), free fatty acids (5), and peroxide value (6), with the spectral groundwork having been laid for the comprehensive assessment of the oxidative state of oils (7). Our overall objective is to develop preprogrammed analytical systems that are capable of carrying out routine edible oil analyses in less than three minutes per sample. In our work to date, the ever-present bottleneck has been the lack of a suitable sample-handling system, as no commercial system is available that can handle both fats and oils. This paper describes a generalized industrial sample-handling platform designed specifically for the analysis of fats and oils and the development of an automated FTIR method for the rapid determination of their *cis* and *trans* contents.

Current Status of trans *Analyses*. The American Oil Chemists' Society (AOCS) officially recognizes three methods (8) for the analysis of *trans* content of fats and oils,

one being specific for margarine. Two of these methods (Cd 17-85 and Ce 1c-89) employ gas chromatography (GC), and the third (Cd 14-61) measures isolated *trans* isomers by conventional IR spectroscopy. Both GC methods allow for the identification of individual *trans* as well as *cis* fatty acids; however, depending on the degree of separation of the various isomers, peak overlap can lead to an underestimation of C18:1*t* (9). Overall, capillary GC is a useful primary method but is not particularly suited to routine quality control applications in a process environment.

The AOCS IR spectroscopic method is based on measuring the peak height of the isolated *trans* band at ~10.3 μm (970 cm^{-1}) and comparing it to that measured in the spectrum of either elaidic acid (when *trans* content > 15%) or methyl elaidate (when *trans* content < 15%) standards. From a practical standpoint, the AOCS method has a number of limitations; i.e., it requires the use of CS_2 (volatile and unpleasant), and saponification and methylation of the oil are required for *trans* contents of <15% because triglycerides exhibit a broad IR absorption band that overlaps with the *trans* band, which affects quantitation (10). Lanser and Emken (11) developed an FTIR method based on measurement of the area of the *trans* peak and demonstrated good agreement with the results obtained by capillary GC. Sleeter and Matlock (12) developed an FTIR procedure for measuring the *trans* content of oils directly in their neat form using a 100-μm KBr transmission cell, eliminating the need for CS_2. Ulberth and Haider (13) used *trans*-free methylated soybean oil mixed with methyl elaidate in combination with spectral subtraction techniques to accurately measure low *trans* contents. PLS chemometric procedures were also used; however, the analytical results were not validated.

Clearly, researchers have been addressing the issue of *trans* determination by FTIR spectroscopy; however, practical sample-handling complications and unfamiliarity of the industry with FTIR methods have effectively stifled the routine application of FTIR spectroscopy to oil analysis. Of these impediments, sample handling is the first hurdle to overcome, as the analysis *per se* and the spectroscopic aspects can readily be automated by macro-programming.

4.3. MATERIALS AND METHODS

Sample-Handling System/Platform. Based on our experience over the past several years in working on FTIR oil analysis, it has become evident that a rugged sample-handling platform is required if FTIR methods are to be routinely employed in quality control of fats and oils. For this purpose, a general fats and oils sample-handling prototype was designed and manufactured in cooperation with Dwight Analytical Solutions Ltd. (Toronto, Ontario, Canada), amenable to "at-line" or laboratory use. Figure 4.1 is a schematic diagram of the integrated system, showing the benchtop FTIR spectrometer, the computer that controls the spectrometer, a temperature controller, and the sample-handling accessory inlet and control valve. Figure 4.2 presents a side view of the accessory within the sample compartment of the spectrometer and shows the cell and its housing, the latter being composed of a temperature control block and a removable cell insert. The insert allows for ready removal of the cell to take an open-beam background or to interchange cells if a change in cell configuration (i.e., windows, pathlength) is required for a particular analysis. The cell insert (not shown) is designed with a spring-loaded face plate, which makes it readily demountable, allowing one to

change windows or spacers as needed. Figure 4.3 presents a schematic diagram of the flow path through the cell and its housing, showing the inlet line, a three-way valve used to direct the oil flow, and an outlet line that empties into a collection vessel (not shown), which in turn is connected to a vacuum line, used to aspirate the sample through the IR transmission flow cell (Dwight Analytical Solutions Ltd., Toronto, Ontario, Canada). All components of the accessory are heated (usually to 80 ± 0.2°C) so that fats will be in their liquid form and flow without crystallization in the lines or cell. The system includes a bypass line to flush out the bulk of the previous sample, allowing rapid sample loading, because the bottleneck of passing large sample volumes through the narrow-pathlength IR cell is avoided, and minimizing sample cross-contamination. Samples are either heated in a test tube block or prewarmed in a microwave oven, presented at the input line, and aspirated through the bypass line and then into the cell via the three-way valve. The system as configured was used throughout this study.

IR Spectroscopy. The instrument employed was a Nicolet Impact 400 FTIR spectrometer interfaced to a 486/33-MHz PC operating under Windows®-based Nicolet® Omnic 2.1 software (Nicolet Instrument Corp., Madison, WI) (14). The instrument and sample compartment were continuously purged with dry air from a Balston dryer (Balston, Lexington, MA) to minimize water vapor and CO_2 interferences. The spectrometer was equipped with the sample-handling accessory described above, operated at 80 ± 0.2°C. A cell with NaCl windows and a nominal pathlength of 25 μm was placed in the cell insert.

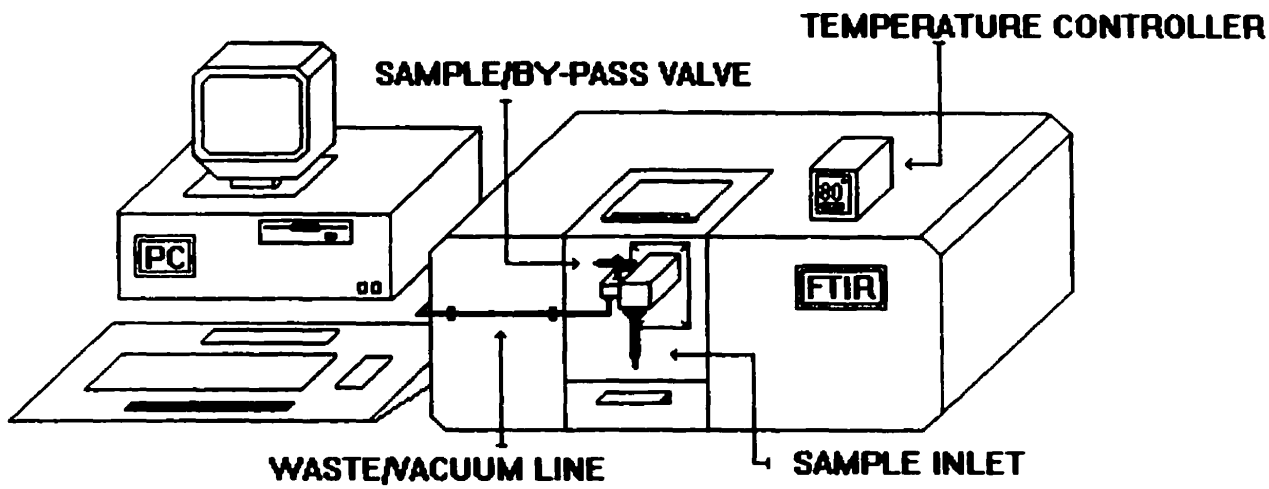

Figure 4.1. A schematic diagram of the FTIR spectrometer, PC, temperature control unit, and oil sample inlet and valve.

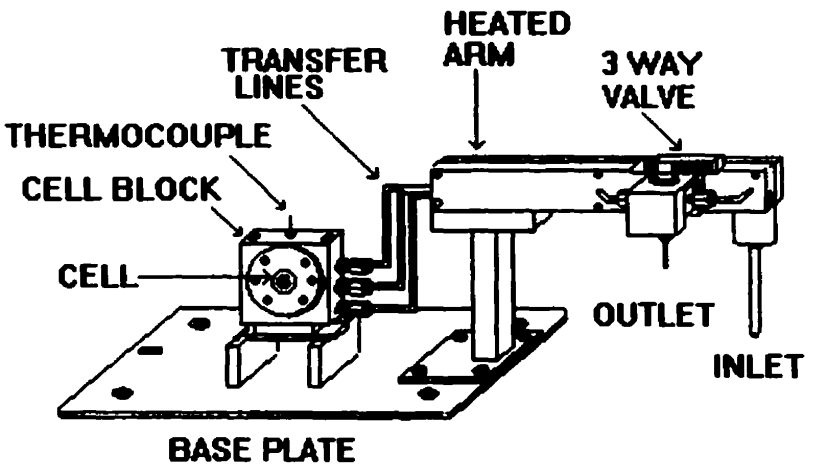

Figure 4.2. Oil analysis accessory (side view), illustrating the cell and heated inlet and outlet lines.

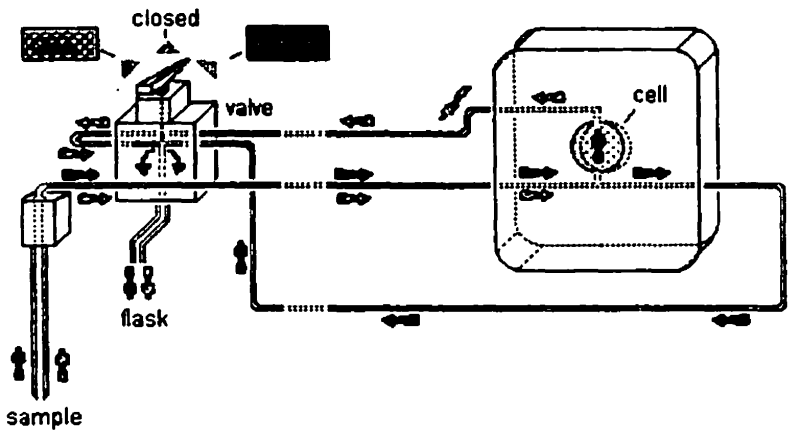

Figure 4.3. A schematic diagram of the cell and flow pattern through the system.

Reagent-grade triglyceride standards (purity >98%) tricaproin (C6:0), tricaprylin (C8:0), tricaprin (C10:0), trilaurin (C12:0), tripalmitin (C16:0), tripalmitolein (C16:1*c*), tristearin (C18:0), triolein (C18:1*c*), trielaidin (C18:1*t*), trilinolein (C18:2*c*), trilinolelaidin (C18:2*t*), trilinolenin (C18:3*c*), and trierucin (C22:1*c*) were obtained from Sigma Chemical Company (St. Louis, MO). These standards were selected to cover a range of molecular weights (SN) and degrees of unsaturation, including both *cis* and *trans* isomers. The *cis* and *trans* contents of the standards were expressed as % triolein and % trielaidin, with C18:1*c* and C18:1*t* representing 100% *cis* and *trans*, respectively, and with C18:2*c*, C18:3*c*, and C18:2*t* having values of 200% *cis*, 300% *cis*, and 200% *trans*, respectively.

Calibration standards were loaded into the cell with a microsyringe because the standards were expensive and available only in limited quantity. The same procedure was employed for samples prepared by standard addition. For the analysis of the other samples, sample handling consisted of warming the sample in a microwave oven to within 5°C of operating temperature and aspirating the sample into the cell (~5 mL to flush the bypass and 2 mL to load the cell), recording its spectrum (128 scans, resolution of 4 cm^{-1} at a gain of 1.0), evacuating the sample, and loading the subsequent sample in the same manner. The cell was only cleaned prior to and at the end of an analytical run with isooctane. Spectra were collected under program control by co-adding 512 scans (calibration standards) or 32 scans (samples).

PLS calibrations were derived for *cis* content, *trans* content, IV, and SN; the basic principles of the calibration have been described in previous publications (4-7). Spectral

mixtures generated from the spectra of the 13 base triglycerides to obtain a wider range of *cis* and *trans* contents than were available from the unsaturated triglyceride standards were also included in the calibrations. The PLS calibrations were validated by using the "leave-one-out" cross-validation procedure and incorporated into a master program, written in Visual Basic, which drove the spectrometer, performed all calculations required, and printed the results. The program also included a Windows-based operator interface, which prompted the user on how to proceed with the analysis.

For the analysis of unknowns, CanAmera Foods Ltd. (Toronto, Ontario, Canada) supplied a selection of vegetable oils, shortenings, and other hydrogenated fats, preanalyzed for IV by the standard AOCS chemical method. FTIR analysis of these samples was performed under program control to provide *cis* and *trans* data directly. The total unsaturation (Σ*cis* + *trans*) was related to IV. Quantitative standard addition was carried out on a micro-scale by adding pure *cis*- and *trans*-unsaturated triglycerides to selected fats and oils to determine whether the calibration responded quantitatively to precise gravimetric changes. For comparative purposes, the instrument was also calibrated by a simple modification of the AOCS method, with pure trielaidin as a standard (100% *trans*), to measure *trans* content from the peak height at 971 cm^{-1} (10.3 μm) relative to a baseline drawn between 995 cm^{-1} (10.05 μm) and 937 cm^{-1} (10.67 μm). This effectively parallels the AOCS method but is suitable for the analysis of oils in their neat form.

4.4. RESULTS AND DISCUSSION

Figure 4.4 presents the overlaid spectra of six triglycerides (C18:0, C18:1*c*, C18:2*c*, C18:3*c*, C18:1*t*, and C18:2*t*) and illustrates the *cis* and *trans* absorption bands. *Trans* fatty acids exhibit a characteristic absorption at ~970 cm^{-1}, whereas *cis* fatty acids are characterized by a distinctive band at ~3010 cm^{-1}, due to the CH stretching absorption of *cis* double bonds. *Trans* fatty acids also exhibit a weaker CH stretching absorption at ~3025 cm^{-1}. Figure 4.5 presents a more detailed view of the variability introduced in the *cis* and *trans* regions when triglycerides are mixed spectrally. This figure illustrates some of the difficulties that one would encounter when using a simple peak height or area measurement to determine the *cis* or *trans* content of an oil. First, the extinction coefficient of the characteristic *trans* band for C18:2*t* is ~1.7 times that for C18:1*t* rather than 2.0 (Figure 4.4). Furthermore, there is a slight shift in the peak maximum on going from C18:1*t* (966.3 cm^{-1}) to C18:2*t* (967.8 cm^{-1}), as illustrated in Figure 4.5B. Thus, in samples containing C18:2*t*, the overall *trans* content would be overestimated by the peak height method. Similar effects are observed for the *cis* band (Figure 4.5A), which shifts from 3004.8 cm^{-1} for C18:1*c* to 3009.8 cm^{-1} for C18:2*c* and 3011.4 cm^{-1} for C18:3*c*. Secondly, in the CH stretching region, the *cis* band partially overlaps the *trans* band, while in the *trans* region, triglyceride contributions interfere with the measurement of the peak height of the *trans* band, and these contributions vary with SN (molecular weight).

PLS is the chemometric method of choice when such interferences are present. The power of PLS is based on its ability to make use of spectral information from broad spectral regions, rather than peak height or peak area measurements, and to

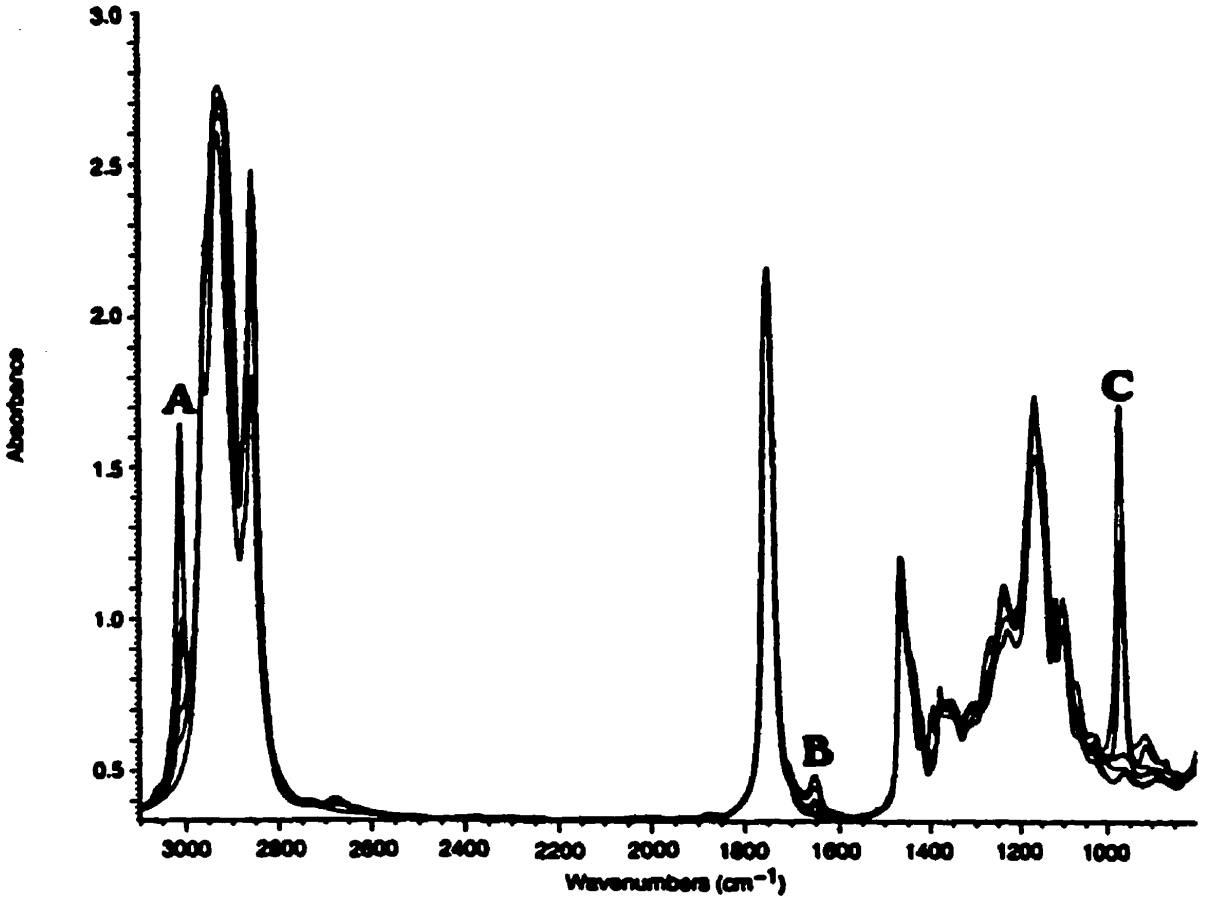

Figure 4.4. Overlaid spectra of C18:0, C18:1*c*, C18:2*c*, C18:3*c*, C18:1*t* and C18:2*t*, illustrating the major bands of interest related to *cis* and *trans* analysis in edible oils: (A) *cis* C-H stretching absorption; (B) C=C stretching absorption; (C) *trans* C=C-H bending absorption.

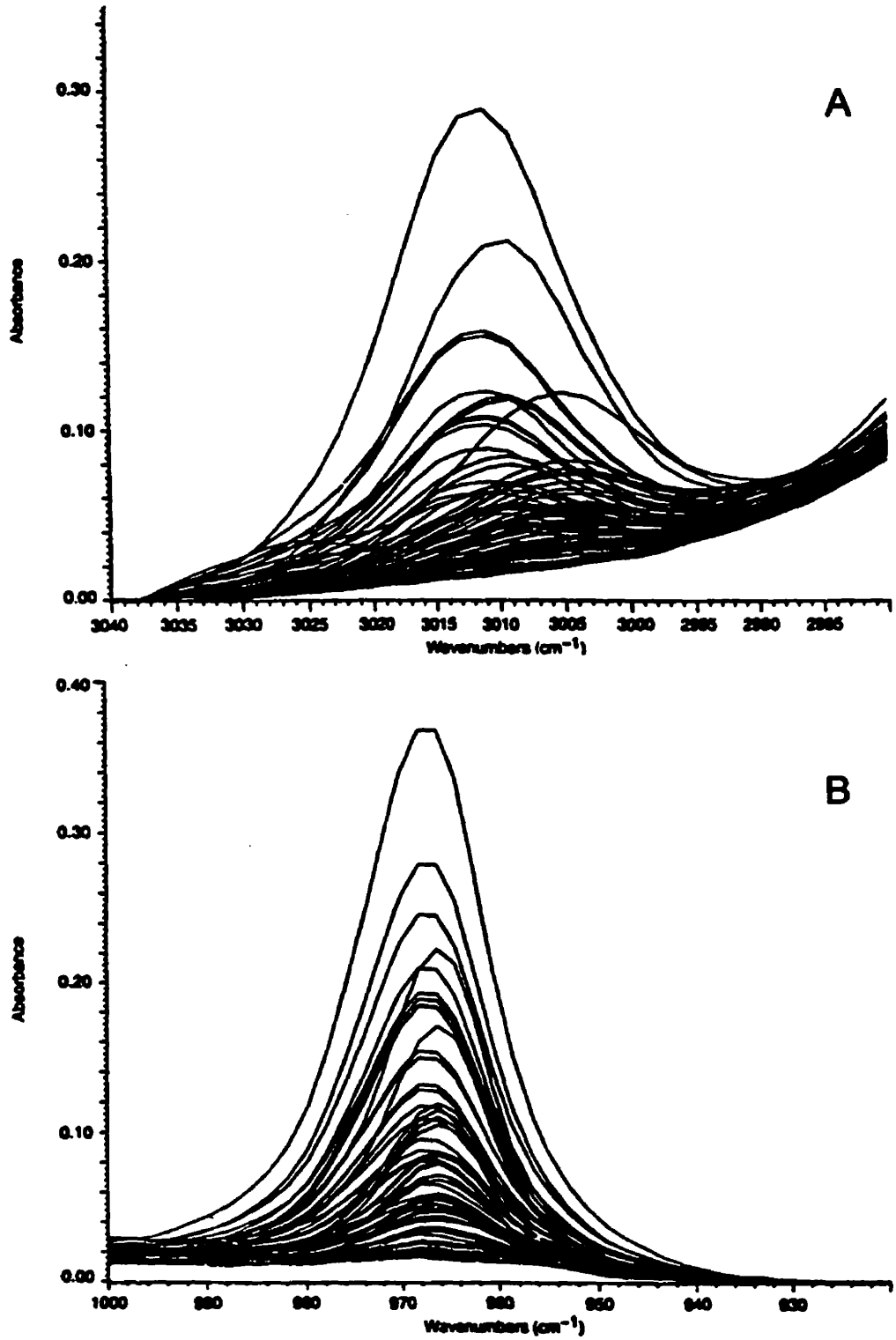

Figure 4.5. Detail of the *cis* (A) and *trans* (B) regions of co-added spectra, illustrating the spectral variability in these regions.

mathematically correlate spectral changes to changes in the concentration of a component of interest, while simultaneously accounting for other spectral contributions that may perturb the spectrum (15). As such, a PLS calibration model is capable of delivering accurate and reproducible results as long as the calibration spectra contain enough information that is representative of both the component of interest and the nonrelated spectral variations associated with the samples to be analyzed. PLS calibration models were developed based on a calibration set that included both C18:1*t* and C18:2*t* and five *cis* isomers (C16:1*c*, C18:1*c*, C18:2*c*, C18:3*c*, and C22:1*c*) as well as six saturated triglycerides that covered a broad range of SNs. This approach allows the spectral shifts between the different *cis* and *trans* forms to be accounted for in the calibrations. Furthermore, as the PLS procedure makes use of broad spectral regions in determining *cis* and *trans* contents, the spectral contribution of the weak *trans* (3025 cm^{-1}) band in the *cis* region is included, as is that of a weak *cis* (913 cm^{-1}) band in the *trans* region. Last, but not least, by incorporating the effect of SN by including saturated triglycerides in the calibration set, the PLS model accounts for the contributions of the underlying triglyceride absorptions that lead to errors in the AOCS method. Our initial calibrations were based on some 70 spectra in all, the 13 base triglyceride spectra plus 57 spectral mixtures produced by co-adding various proportions of the base spectra to provide a wider range of *cis* and *trans* values. The additional information generated from the co-added spectra was found to be redundant in improving the calibration, and the 13 base standards sufficed. However, the co-added spectra were useful for comparative, illustrative, and interpretational purposes. Excellent calibrations were obtained for *cis* and *trans* determinations as well as for SN and IV (not discussed). These calibrations were

validated by the "leave-one-out" cross-validation technique, with the mean errors obtained from the cross validation being 0.10 and 0.05% for *cis* and *trans*, respectively.

Analyses were initially carried out on commercially available fats and vegetable oils to test the sample-handling system. The sample-handling prototype proved to be functional, rugged, reliable, and easy to use, effectively overcoming all the common sample-handling problems encountered in our previous work. At 80°C, even hardened fats were liquid and flowed well through the system. Flow through both the bypass line and the cell was smooth, and no cell loading or cross-contamination problems were encountered. Table 4.1 provides an assessment of between-run precision (16) in terms of the mean difference (MD_r) and the standard deviation of the differences (SDD_r) for *cis*, *trans*, IV, and SN predictions for 20 sample pairs run one week apart. Consistent results were obtained in terms of the overall means, regardless of the sample, with the variability around the mean difference being about ±0.25% for the *cis* and *trans* data. This type of consistency was the norm and could be maintained over time.

Subsequent experiments involved standard addition of *cis* and *trans* triglycerides in milligram amounts to small amounts of selected oils to determine if changes in *cis* and *trans* content were accurately quantified, in effect a measure of the accuracy of the analysis. Table 4.2 presents the data obtained in terms of accuracy (MD_a and SDD_a) for the standard addition experiments. The standard addition results indicate that, on average, the predictions matched the calculated values quite well. Subsequently, similar experiments were carried out by mixing selected oils in bulk in gravimetrically precise proportions to eliminate the sources of variability associated with weighing and mixing

Table 4.1. MD_r and SDD_r for 20 Sample Pairs of Various Oils Run One Week Apart

Parameter	MD_r	SDD_r
cis content	-0.69	0.23
trans content	0.14	0.23
IV[a]	-0.77	0.52
SN[a]	-0.19	0.22

[a]For information only, not discussed.

Table 4.2. MD_s and SDD_s for 15 Samples[a]

Parameter	MD_r	SDD_s
cis content	0.30	1.31
trans content	-0.69	1.25
IV[b]	0.48	1.61
SN[b]	0.06	0.53

[a]The FTIR predictions are compared to calculated change expected in the values based on the standard addition of pure *cis/trans* triglycerides.
[b]For information only, not discussed.

on the small scale required for standard addition and loading the cell with a microsyringe. Similar results were obtained, indicating that the standard addition results were representative of the performance one could expect from FTIR analysis. Hence, the PLS calibration was shown to respond quantitatively to the direct addition of *cis* and *trans* moieties to diverse oils, as well as to the mixing of different fats and oils. These results imply that the spectra of only 13 pure triglycerides provide sufficient information to quantitate complex triglyceride systems in terms of their *cis* and *trans* contents.

To further determine the universality of the calibration, additional FTIR analyses were carried out on 31 fats and oils, supplied and independently analyzed for their IV by CanAmera Foods Ltd. The resulting *cis* and *trans* predictions are tabulated in Table 4.3 along with their $\Sigma cis + trans$ plus their chemical IV. Because *cis* and *trans* data were not available for these samples, IV was used as the basis for assessing the accuracy of the FTIR-predicted values. IV represents the sum of *cis* and *trans* double bond contributions and can be expressed in terms of % triolein and % trielaidin as follows:

$$IV = 3[(C + T)\,(Mw\ I_2/Mw\ TG)] \qquad [4.1]$$

$$= 0.8601(C + T) \qquad [4.2]$$

where:
- C = % *cis* (as triolein)
- T = % *trans* (as trielaidin)
- Mw = Molecular weight
- TG = Triglyceride (triolein or trielaidin)

Based on this relationship, one would expect the FTIR-predicted $\Sigma cis + trans$ to be linearly related to the chemical IV if the instrumental predictions are on track. Figure 4.6 presents a plot of $\Sigma cis + trans$ versus IV for the data presented in Table 4.3. This plot illustrates that there is a good linear correspondence between the FTIR-predicted

Table 4.3. FTIR-Predicted *cis* and *trans* Contents and Chemical Iodine Values for Samples of Fats and Oils

Fat or oil[a]	% *cis*	% *trans*	Σ C/T	Chem IV
Corn	148.7	0.1	148.8	128.1
Olive	92.6	3.4	96.0	86.9
Safflower	167.4	0.9	168.3	146.6
Lard 1	69.6	2.0	71.6	60.1
Sesame	130.3	2.4	132.7	110.6
Coconut	11.3	-2.0	9.3	8.7
Peanut	109.4	0.2	109.6	95.6
Lard 2	75.4	1.7	77.1	62.0
Cottonseed	123.2	0.5	123.7	105.7
Canola	131.7	0.5	132.2	116.0
Soya	151.0	0.0	151.0	129.3
Tallow	45.4	7.2	52.6	43.2
Palm Kernel Oil	22.0	- 2.5	19.5	17.3
Sample 870 ST-4	49.8	34.4	84.2	72.7
Sample 869 ST-5	116.5	14.2	130.7	109.6
Sample 469 ST-6	64.6	31.9	96.5	80.7
Sample 460 ST-6	36.0	44.5	80.5	71.0
Sample 866 ST-4	115.5	13.9	129.4	107.3
Sample 448 ST-5	45.8	41.7	87.5	75.3
Sample 473 ST-8	39.5	43.0	82.5	69.5
Sample 865 ST-5	98.9	21.0	119.9	102.2
Sample 864 ST-9	42.0	40.7	82.7	71.2
Majestic ST-8	49.7	36.5	86.2	74.0
RH 26039	52.0	37.3	89.3	76.8
ST-9	46.0	6.4	52.4	48.3
ST-7	59.2	32.0	91.2	77.7
ST-6	50.6	40.1	90.7	77.3
K	42.2	44.5	86.7	70.6
PBY 18844	38.4	46.0	84.4	73.0
EFFEM	36.5	46.2	82.7	74.3
JP	40.1	39.9	80.0	70.3

[a]All samples supplied by CanAmera Foods Ltd. (Toronto, Canada); coded samples were of unspecified composition.

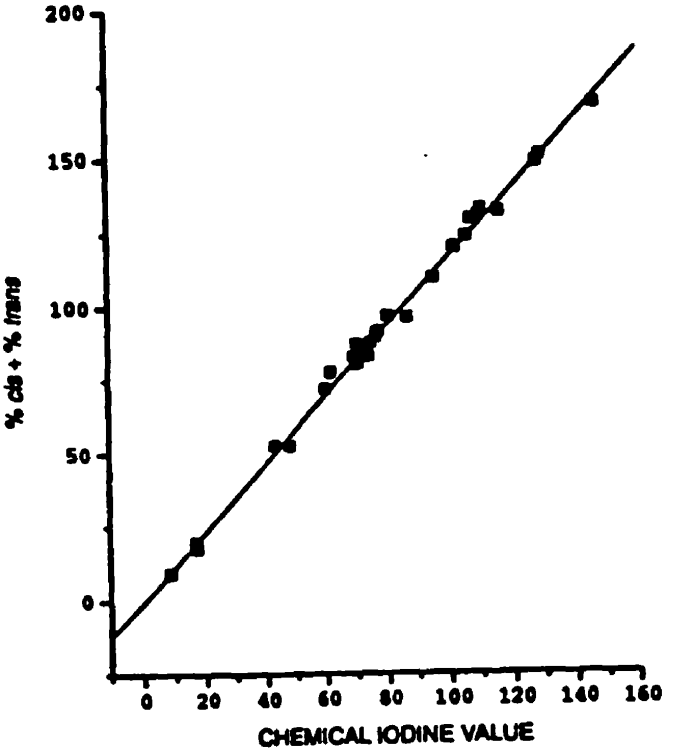

Figure 4.6. Plot of the total degree of unsaturation (*cis* + *trans*) for the 29 CanAmera samples as determined by FTIR analysis vs. their chemical IV.

Σcis + *trans* and IV, with linear regression of these data producing the following relationship:

$$\Sigma cis + trans = -0.523 + (1.172)IV \qquad r^2 = 0.997 \qquad SD = \pm 2.60\% \qquad [4.3]$$

The overall error of ~2.5% in terms of accuracy is reasonable because it includes the experimental error associated with the IV analysis itself plus that associated with the *cis* and *trans* predictions. The inverse of the slope of the plot (0.853) is in line with the theoretical conversion factor of 0.8601 (Eq. [4.2]).

In Table 4.3, the range of *trans* content runs from negligible to ~46%. The only apparent problem with the data is some negative predictions associated with samples having high saponification numbers (palm and coconut). A similar, but more muted, tendency was seen in predictions of spectral mixtures of the calibrations standards that represent *trans* values of zero, specifically mixtures corresponding to saponification numbers of >250 (average chain lengths of ~C_{12}–C_{14}). To determine how the AOCS method would perform in this regard, the 70 ideal spectral mixtures were analyzed by the modified AOCS method, using trielaidin as a standard, and the results were plotted versus the theoretical values for the spectral mixtures. Figure 4.7 shows only the lower portion of this plot (0-30% *trans*) and illustrates the effect that the SN of saturated triglycerides (zero *trans*) has on their predicted *trans* values. As such, the AOCS method cannot differentiate between fully saturated lipids and lipids with low *trans* contents, requiring the use of the methylation procedure for samples with *trans* contents of <15%. Methylation is not required in the PLS approach, which does well down to ~1% *trans*. Figure 4.8 shows a plot of the PLS-predicted values for the CanAmera samples versus the

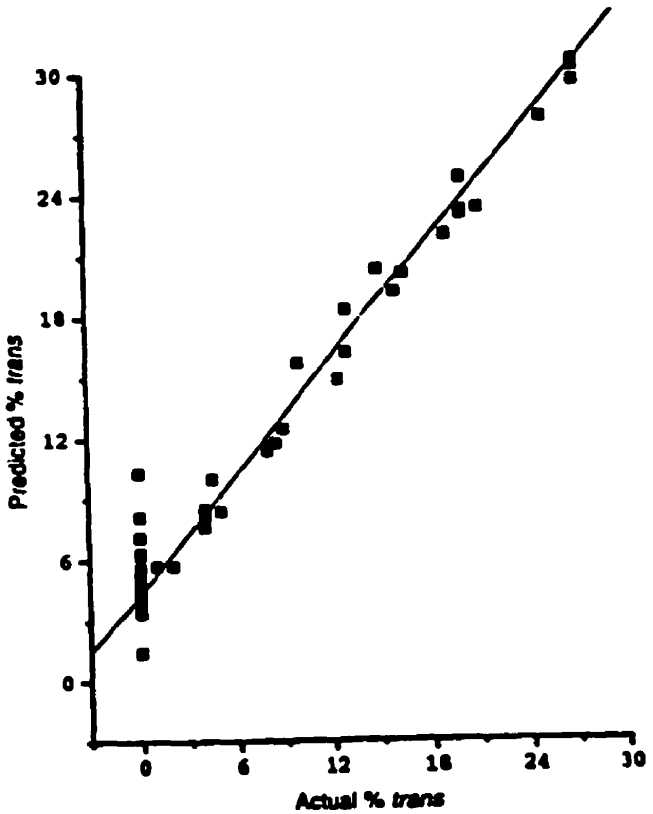

Figure 4.7. Plot of predicted % *trans* obtained for co-added spectral mixtures of the base triglycerides by the modified AOCS method using trielaidin as the standard vs. the theoretical % *trans*.

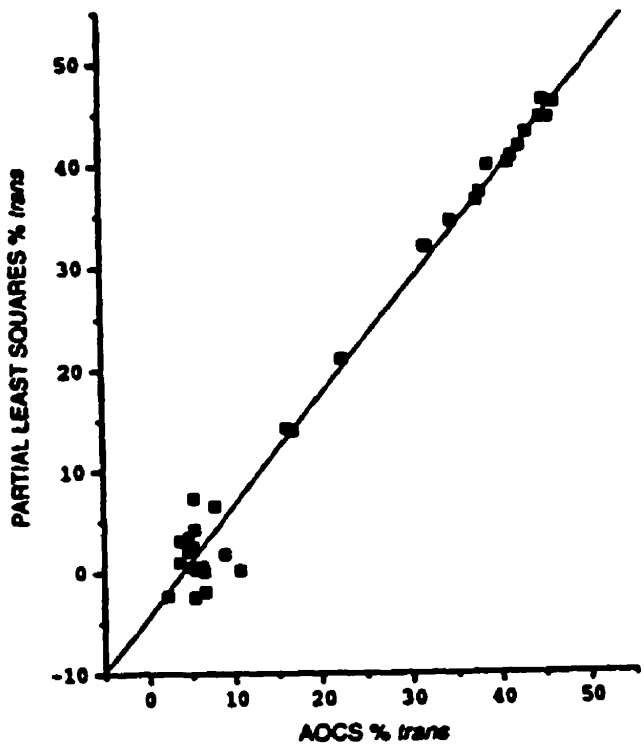

Figure 4.8. Plot of predicted % *trans* obtained for the 29 CanAmera samples by the PLS-FTIR method vs. the predictions obtained from the modified AOCS method using trielaidin as the standard.

values obtained by analyzing the same spectral information using the modified AOCS method with trielaidin as a standard. A good linear relationship holds between the FTIR-PLS and FTIR-AOCS *trans* predictions above values of ~12%, but the relationship breaks down at lower *trans* values. Below 12% *trans*, the AOCS method again becomes limiting because it cannot account for underlying triglyceride absorptions. In the case of the PLS calibration, on the other hand, the Σ*cis* + *trans* continues to correlate with the chemical IVs close to 0% *trans*. Hence, there is ample evidence that the PLS calibration is more robust and has fewer sources of variability and error than the AOCS method. In addition, the FTIR-PLS method is advantageous in terms of speed and accuracy and is able to determine both *cis* and *trans* contents, as well as IV and SN, in a single analysis.

As structured, the FTIR method makes use of a generalized sample-handling accessory designed specifically for fats and oils. Sample handling is straightforward, and because the calibration is based on 13 commercially available triglycerides, the calibration is applicable to refined triglyceride-based oils in general, barring any extensive degree of oxidation. Not discussed here are calibration transfer concepts which have been developed to allow calibrations to be transferred between instruments, nor the programs written to automate the analysis. These additional elements of the analytical system effectively eliminate the need for calibration or for an operator to have any knowledge of FTIR spectroscopy. As such, the system is designed for industrial "at line" use in an oil-processing environment, where it would allow for the simultaneous determination of *cis* and *trans* content, IV, and SN within a total analysis time of 1-2 min. This system is the first step in the development of an integrated sample-handling platform

for FTIR edible oil analysis, with transferable calibrations, computerized automation, and the appropriate use of chemometrics. Such a system provides an opportunity for replacing a host of tedious AOCS wet chemical methods, many of them of starting to be affected by environmental concerns.

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BRIDGE

The validation studies described in the previous chapter included an evaluation of the results that would be obtained from the analysis of neat fats and oils or pure triglycerides by the traditional peak height-based IR method for the determination of isolated *trans* isomers. Comparison between the *trans* values obtained from this method and those predicted from a PLS calibration model illustrated the extent to which the peak height method is subject to interference from underlying triglyceride absorptions when samples having *trans* levels of <15% are analyzed. In the traditional AOCS method, oils are analyzed as fatty acid methyl esters in order to eliminate these interfering absorptions. In the following chapter, the use of the spectral ratioing capability of FTIR spectroscopy as a means of eliminating the need to saponify and methylate the oil is reported. This work provided a *trans* peak height-based method, designed for use with the transmission flow cell sample-handling accessory described in Chapter 4, that allows fats and oils to be analyzed in their neat form.

CHAPTER 5

UPGRADING THE AOCS IR *TRANS* METHOD FOR ANALYSIS OF NEAT FATS AND OILS BY FTIR SPECTROSCOPY

5.1. ABSTRACT

An automated protocol for the direct, rapid determination of isolated *trans* content of neat fats and oils by FTIR spectroscopy was devised, based on a simple modification of the standard AOCS *trans* method, eliminating the use of CS_2 and methylation of low-*trans* samples. Through the use of a commercially available, heated transmission flow cell, designed specifically for the analysis of neat fats and oils, a calibration (0-50% *trans*) was devised with trielaidin spiked into a certified, *trans*-free soybean oil. The single-beam spectra of the calibration standards were ratioed against the single-beam spectrum of the base oil, eliminating the spectral interference caused by underlying triglyceride absorptions, facilitating direct peak height measurements as per the AOCS IR *trans* method. The spectrometer was preprogrammed in Microsoft® Visual Basic to carry out all spectral manipulations, measurements, and calculations to produce *trans* results directly as well as to provide the operator with a simple interface to work from. The derived calibration was incorporated into the software package, obviating the need for further calibration because the program includes an automatic recalibration/ standardization routine that automatically compensates for differences in optical characteristics between instruments, instrument drift over time, and cell wear. The modified AOCS FTIR analytical package was evaluated with Smalley check samples for repeatability, reproducibility, and accuracy, producing SDs of ± 0.07, 0.13, and 0.70

percent *trans*, respectively, the FTIR predictions being linearly related to the Smalley means (r = 0.999; SD = ± 0.46) and well within one SD of the Smalley sample means. Calibration transfer was assessed by implementing the calibration on a second instrument and reanalyzing the Smalley check samples in cells of two different pathlengths (25 and 50 μm). There were no statistically significant differences between the FTIR *trans* predictions obtained for the Smalley samples from the two instruments and two cells, indicating that the software was able to adjust the calibrations to compensate for differences in instrument response and cell pathlength. The FTIR isolated *trans* analysis protocol developed by the McGill IR Group has the benefit of being based on the principles of an AOCS-approved method, matches its accuracy, and allows the analysis to be performed on both neat fats and oils, producing *trans* predictions in less than 2 min per sample. It is suggested that this integrated approach to *trans* analysis, which requires a minimum level of sample manipulation and operator skill, be considered as a modification of the proposed Recommended Practice Cd 14b-95.

5.2. INTRODUCTION

Edible fats and oils vary in their triglyceride makeup, relative degree and forms of unsaturation (*cis* and *trans*), weight-average molecular weight, and overall fatty acid composition and distribution, and these complex determinants define the physico-chemical properties of the lipid system. For stability and functionality reasons, oils are often hydrogenated and converted into fats. Hydrogenation reduces the overall degree of unsaturation and leads to increased levels of *trans* fatty acids, now of increased concern to nutritionists owing to their association with heart disease (1). *Trans* fatty acid levels

can reach 40% or more in hardened fats, and the U.S. Food and Drug Administration has been petitioned to require the inclusion of *trans* fatty acids as part of saturated fat content in the labeling of fat-based products, such as margarines, spreads, and frying fats (2, 3). *Trans* analysis by IR spectroscopy was developed over 40 years ago with dispersive IR instrumentation and is a well-established AOCS official method (4). The limitations of the method have been well documented, specifically the fact that the method is not applicable to oils that contain conjugated *trans/trans* and *cis/trans* bonds, requires the use of a volatile and noxious solvent (CS_2), and is affected by overlap of the *trans* peak by underlying triglyceride absorptions, which contribute significantly to the *trans* peak height measurement at low *trans* levels (<15%). To eliminate the interfering triglyceride absorptions, the oil must be saponified and methylated. The official methodology has recently been updated (5) to take advantage of FTIR instrumentation and computerized data analysis techniques; however, the inherent limitations noted above remain.

The McGill IR Group has been carrying out research into the development of simple, rapid, and accurate methods of edible oil analysis based on FTIR spectroscopy (6-13). One of the practical developments of this work has been an FTIR-based edible oil analysis package, which is capable of simultaneously determining iodine value (IV), saponification number (SN), and *cis* and *trans* content (IV/SN/*cis*/*trans*) in a single analysis on a neat fat or oil sample in less than 2 min (11). The system is preprogrammed and precalibrated and uses a heated sample-handling accessory (11) to allow both fats and oils to be analyzed in their liquid state at 80°C. This system is being used by a number of edible oil processors; however, the fact that it employs a relatively sophisticated

multicomponent analysis approach based on partial-least-squares (PLS) regression, the principles of which are not well understood by most users, may hinder its general acceptance. For many analysts in industry and regulatory agencies, reference to an approved methodology is highly desirable and often a requirement; hence, there is a place for a simplified, improved, and automated technique based on the well-defined and accepted analytical concepts associated with the official AOCS *trans* method. This paper describes an updated and automated version of the AOCS *trans* analysis procedure that eliminates the need for CS_2 and methylations, by taking advantage of the spectral ratioing capability of FTIR spectroscopy and making use of a dedicated sample-handling accessory, and allows the *trans* analysis to be carried out in less than 2 min per sample.

5.3. MATERIALS AND METHODS

Instrumentation/Spectral Acquisition. The instruments used were Nicolet Magna and Protégé FTIR spectrometers, controlled by 486 PCs running under Nicolet's Omnic 3.0 software; the two instruments utilized the same type of source (Globar) and detector [deuterated triglycine sulfate (DTGS)]. Each instrument was equipped with a heated sample-handling accessory (Figure 5.1) manufactured by Dwight Analytical (Toronto, Ontario, Canada) and fitted with either a 50- or a 25-μm KCl flow cell maintained at 80°C. Both spectrometers were continuously purged with CO_2-free dry air, supplied by a Balston dryer (Balston, Lexington, MA), to minimize spectral interferences from water vapor and carbon dioxide. All spectra were collected by co-adding 64 scans (calibration standards) or 32 scans (samples) recorded at a resolution of 4 cm^{-1} and a gain of 1.0.

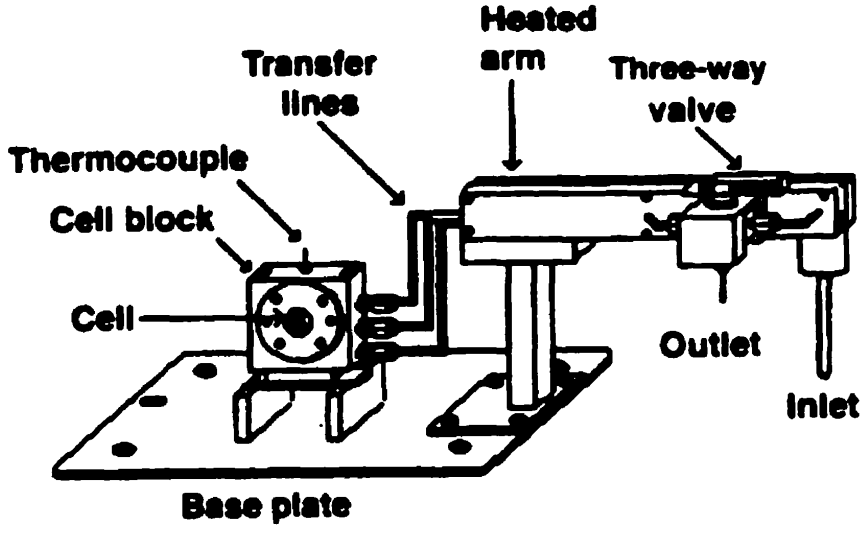

Figure 5.1. Schematic diagram of the heated transmission flow cell and sample-handling accessory used for the analysis of neat fats and oils.

Calibration. A *trans* calibration set was devised by gravimetrically spiking trielaidin (Sigma Chemical Co., St. Louis, MO) into an unhydrogenated soybean oil with a *trans* content of <0.1% as determined by GC (AOCS Method Ce 1c-89), to cover a range of 0-80% trielaidin. The calibration was performed by recording the spectra of these standards in a 25-μm cell inserted into the sample-handling accessory (maintained at 80°C) installed in the compartment of the Magna spectrometer and ratioing the spectrum of each standard against that of the *trans*-free base oil to produce a "differential spectrum." The concentrations of trielaidin in these standards were related to the peak height measured in the differential spectra at 966 cm^{-1}, corresponding to the position of the peak maximum of trielaidin in the spectra of the calibration standards, relative to a baseline drawn between 995 and 937 cm^{-1}. A calibration equation was derived by linear regression of peak height versus the gravimetric *trans* content added to the base oil. A program was written in Microsoft® Visual Basic to carry out peak height measurements and calculate the *trans* content by using the calibration equation derived. In addition, the program developed for the analysis included a simple menu to guide the operator in collecting the spectra, as well as calibration diagnostics and automatic recalibration routines.

Samples/Analyses. To assess the performance of the modified AOCS method, seven Smalley *trans* check samples were obtained from the 1995 series, for which results were already available. These samples were analyzed by FTIR spectroscopy using our preprogrammed ratio approach, here termed the modified AOCS FTIR method. The spectra of all samples analyzed were ratioed against the same *trans*-free soybean oil

spectrum as employed in deriving the calibration. Prior to analysis, the samples were prewarmed in a microwave oven to ~80°C to melt any solids and aspirated into the IR cell. The samples were run in duplicate to determine repeatability (back-to-back scans of the same sample) and reproducibility (two consecutive loadings). Accuracy was evaluated by comparing the FTIR results to the Smalley sample means, the paired data sets being assessed in terms of mean difference (MD) and standard deviation of the differences (SDD) (14). In subsequent experiments, calibration transfer was assessed by analyzing the Smalley check samples on a second spectrometer (Protégé) with two cells of different pathlengths (50 and 25 μm). The purpose was to test the ability of the software to compensate for both instrument and pathlength changes. After each calibration adjustment for any instrument or cell configuration change, the Smalley test samples were run and the results collated and subjected to analysis of variance (ANOVA) to determine if there were any significant differences between the results obtained with different instruments and/or cell pathlengths. In addition, the *trans* values predicted with the IV/SN/*cis*/*trans* analysis package were compared to the data obtained with the modified AOCS FTIR method.

Spiking Experiments. Canola, corn, coconut, soybean, and sunflower oil were purchased locally. These oils were gravimetrically spiked with trielaidin at levels of 1.5-7% and analyzed as described above.

5.4. RESULTS

Figure 5.2A shows the *trans* absorption region (995-937 cm^{-1}) in the overlaid spectra of the standards employed to derive the calibration equation for the modified

AOCS method, prepared by adding varying amounts of trielaidin to a *trans*-free soybean oil. The corresponding spectra obtained by ratioing the single-beam spectra of these calibration standards against the single-beam spectrum of the base oil are presented in Figure 5.2B. The latter illustrate the horizontal baseline produced by the ratioing procedure, which eliminates some of the uncertainty in the measurement of the *trans* peak height. The standard curves derived from the spectra shown in Figures 5.2A and 5.2B are presented in Figures 5.3A and 5.3B, respectively. These plots are reasonably linear, although we and others (15) have noted that there is significant curvature beyond 50% *trans* and that accurate analysis beyond this value requires a quadratic fit. The equations obtained by simple linear regression over the range of 0-50% *trans* are presented in Eqs. [5.1] and [5.2], respectively.

$$\%\ trans = -3.917 + 131.276\ A_u(966) \qquad R = 0.999 \qquad SE = 0.430 \qquad [5.1]$$

$$\%\ trans = -0.230 + 130.973\ A_r(966) \qquad R = 0.999 \qquad SE = 0.398 \qquad [5.2]$$

where: % *trans* = *trans* content expressed as % trielaidin

$A_u(966)$ = absorbance at 966 cm^{-1} relative to a baseline drawn between 995 and 937 cm^{-1} (spectrum ratioed against air)

$A_r(966)$ = absorbance at 966 cm^{-1} relative to a baseline drawn between 995 and 937 cm^{-1} (spectrum ratioed against reference oil)

Equation [5.1] shows that the underlying triglyceride absorption in the spectrum of the *trans*-free oil corresponds to an intercept value of ~4% *trans*, whereas the intercept is eliminated from the calibration equation obtained by using the ratioing procedure. Equation [5.2] was incorporated into a Visual Basic program to allow for the direct

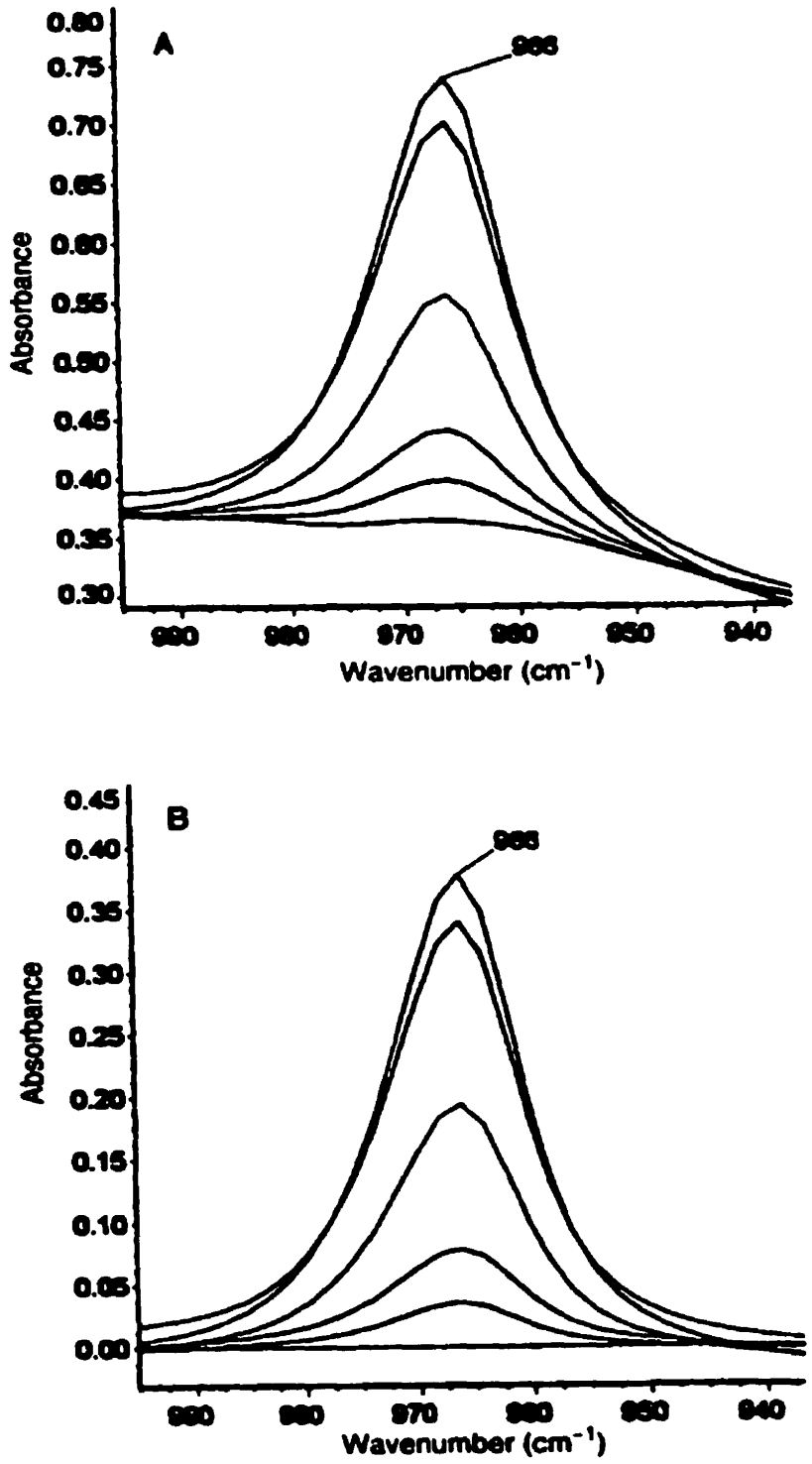

Figure 5.2. (A) Overlaid spectra in the *trans* absorption region of calibration standards prepared by addition of various amounts of trielaidin to a *trans*-free soybean base oil; (B) same spectra as in (A) after ratioing out the spectrum of the base oil.

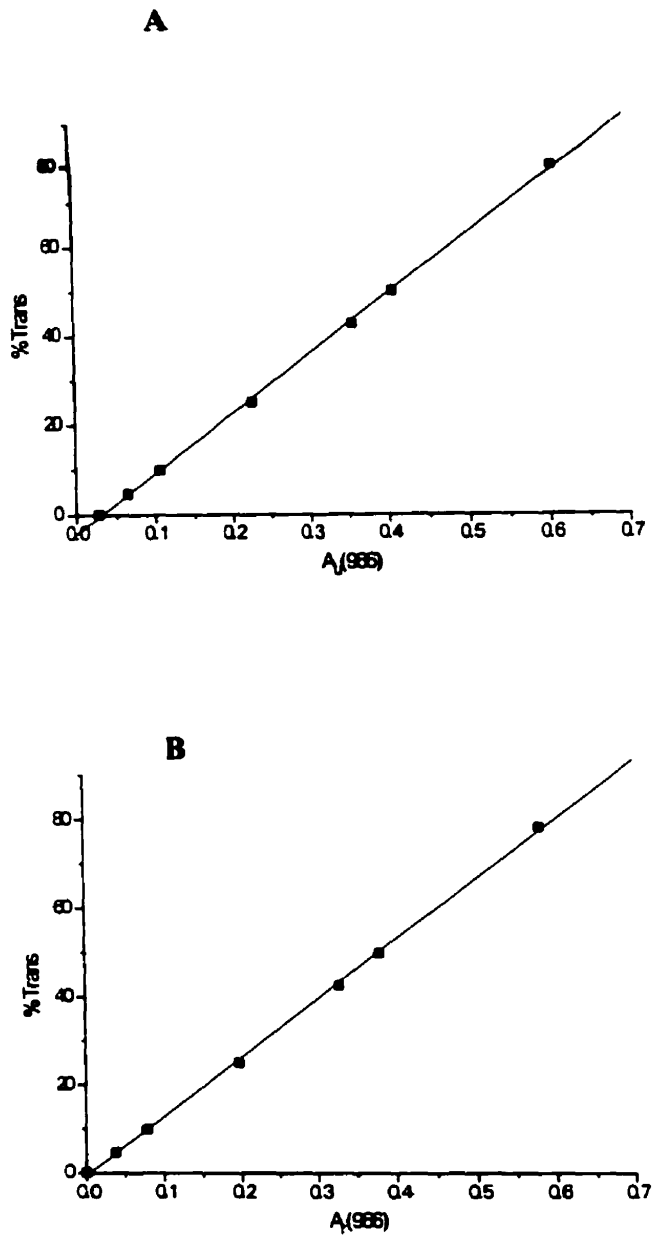

Figure 5.3. Calibration curves obtained for trielaidin in a *trans*-free oil, illustrating the removal of the intercept caused by underlying absorptions when the spectra are ratioed against the spectrum of the *trans*-free oil. (A) Spectra ratioed against an air background spectrum; (B) spectra ratioed against the spectrum of the *trans*-free oil. These plots have some curvilinearity and are better fitted using a quadratic function if concentrations above 50% are considered.

prediction of *trans* values of neat fat or oil samples loaded into the IR cell. The program also included calibration update routines to adjust the calibration equation in order to compensate for changes in cell pathlength or instrument response.

5.4.1. Repeatability and Accuracy of the FTIR Data

One of the recurrent problems with validating *trans* methodology is obtaining reference samples with *trans* values that one can have confidence in. Because there are no absolute standards *per se*, other than pure *trans* triglycerides, it was determined that the best approach would be to rely on the AOCS Smalley check samples, which provide a range of *trans* levels (~0-40% *trans*) and have been analyzed by a number of laboratories (15-18 laboratories, depending on the samples in question) by using the conventional AOCS IR method. After outlier removal, the average *trans* values provide a benchmark value for each sample as well as a standard deviation around its mean. Using the seven samples available, the repeatability and accuracy of our modified AOCS method were evaluated in terms of MD and SDD (Table 5.1).

In terms of repeatability, duplicate analyses show no bias relative to each other in terms of MD, which ideally would be zero. The SDD, a measure of the variability around the MD, is less than ±0.1 for two consecutive scans, while for two separate loadings of the same sample, it rises to ±0.13. These data provide an assessment of the stability of the FTIR data and are an indication of excellent temperature control. To evaluate accuracy, the means of the repeatability runs were compared to the means reported by the AOCS for the Smalley check samples. The MD_a is close to zero and the SDD_a is ±0.7. Figure 5.4 presents a plot of our FTIR results versus the Smalley check sample means.

Table 5.1. Raw Data for Repeatability and Accuracy for *trans* Analysis of the Smalley Check Samples by the Modified AOCS FTIR Method[a]

Smalley sample number	Consecutive scans[b]		Consecutive loadings[b]		Accuracy[b]	
	FTIRa	FTIRb	FTIR1	FTIR2	FTIR12	Smalley
1	16.2	16.3	16.3	16.1	16.20	15.98
2	0.0	0.1	0.0	0.0	0.00	1.37
3	3.9	3.9	4.0	3.9	3.95	4.07
4	8.5	8.6	8.6	8.4	8.50	8.83
5	16.3	16.3	16.4	16.1	16.35	16.19
6	32.3	32.2	32.4	32.0	32.20	31.38
7	40.7	40.7	40.8	40.6	40.70	40.29
Mean	16.84	16.87	16.93	16.73	16.84	16.87
MD	-0.03		0.20		0.03	
SDD	0.075		0.129		0.696	
CV	0.40%		0.76%		4.30%	

[a]Statistical comparisons are summarized in terms of overall mean for each sample as well as mean difference (MD), standard deviation of the differences (SDD), and coefficient of variation (CV).

[b]Data obtained using 25-μm cell on Magna spectrometer.

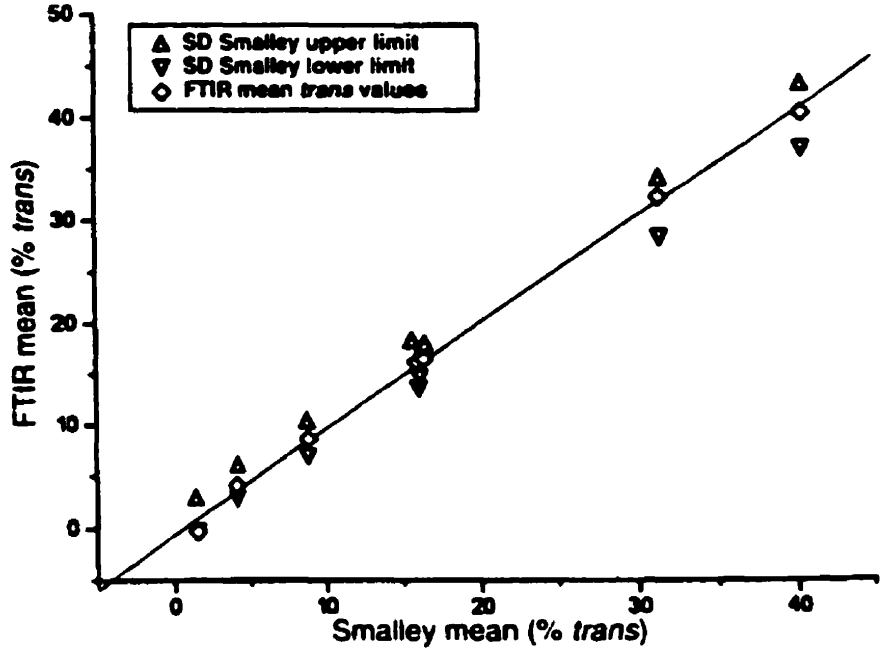

Figure 5.4. A plot of the FTIR predictions for the Smalley check samples obtained using the modified AOCS method vs. the Smalley means, with the triangles above and below the regression line representing one SD around the Smalley means.

The Smalley means plus their SD as well as the means minus their SD are also plotted on the *y* axis for comparison to illustrate the spreads around the Smalley means.

Figure 5.4 illustrates that there is an excellent linear relationship between the FTIR means relative to the Smalley check sample means. The data pass through the center of the Smalley SD limits, with the exception of the lowest value, which was determined to be zero *trans* by the modified FTIR method vs. the Smalley mean value of 1.37% *trans*. The equation for the line was

$$\text{FTIR \% } trans = -0.602 + 1.038 \text{ Smalley \% } trans \qquad r = 0.999 \qquad SE = 0.478 \qquad [5.3]$$

These analytical data provide evidence that the FTIR methodology developed produces results that fall within one SD of the mean values obtained by other laboratories that carried out the same analysis by the traditional AOCS method.

Subsequent series of analyses of the Smalley check samples were carried out on a second spectrometer with two cells of two different pathlengths to test the recalibration and standardization routines associated with the analytical software package as well as to assess the between-run precision of the analysis under variable conditions. The results of these analyses, as well as the corresponding *trans* results obtained using the PLS-based IV/SN/*cis*/*trans* analysis package, are presented in Table 5.2. The means obtained for instrument 2 are similar to those for instrument 1, presented in Table 5.1. ANOVA and the Duncan Multiple Range test indicated that there were no significant differences ($P < 0.01$) between the results obtained for the two instruments or the two cells, indicating that the base calibration produces consistent results when the calibration is updated. The *trans*

Table 5.2. Smalley Check Sample FTIR Results Obtained by Transferring the Calibration Equation Obtained with a 25-μm Cell on a Magna Spectrometer (Instrument 1) to a Protégé Spectrometer (Instrument 2) with 50- and 25-μm Cells[a]

Smalley sample number	Modified AOCS Instrument 1 25-μm Cell	Modified AOCS Instrument 2 25-μm Cell	Modified AOCS Instrument 2 50-μm Cell	PLS FTIR Instrument 2 25-μm Cell	Smalley mean
1	16.2	16.3	15.7	15.3	15.98
2	0.0	0.0	0.0	0.0	1.37
3	3.9	4.0	3.7	3.8	4.07
4	8.5	8.5	8.2	8.0	8.83
5	16.3	16.3	15.9	15.5	16.19
6	32.2	32.2	31.7	32.2	31.38
7	40.7	41.7	40.3	40.4	40.29
Mean	16.84	17.00	16.50	16.46	16.87

[a]These data are compared to the *trans* values obtained using a PLS-based calibration model as well as the Smalley mean *trans* values.

data produced by the PLS multicomponent analysis package also were not significantly different from the data produced by the peak height method. These results corroborate our previous conclusion that the PLS approach produces *trans* values that are in agreement with those obtained by the modified FTIR AOCS method (16).

5.4.2. Spiking Experiments

The accuracy of the *trans* predictions obtained from the FTIR ratioing method is expected to depend to some extent on how closely the triglyceride composition of the samples being analyzed resembles that of the *trans*-free reference oil employed, because this will affect the accuracy of the ratioing out of the underlying triglyceride absorptions. Accordingly, the validation study with the Smalley check samples (hydrogenated soybean oils) described above represents the optimal situation in which the samples are of the same oil type as the reference oil. To assess the analytical accuracy obtained with other types of oils, we prepared a set of samples by spiking pure trielaidin into five different oils (canola, coconut, corn, soybean, and sunflower) at levels of 1.5-7%. The samples were then analyzed by the FTIR ratioing method in the same manner as described above for the Smalley check samples; i.e., the *trans*-free soybean oil was used as the reference oil and the calibration equation derived using the trielaidin-spiked soybean standards (Eq. [5.2]) was employed. The FTIR *trans* predictions for all these spiked samples were within one percentage point of the gravimetric values, demonstrating that the ratioing procedure eliminated the bulk of the contribution of the underlying triglyceride absorptions, even though the five oils employed in these spiking experiments vary widely in triglyceride composition. The *trans* content of each of the spiked samples was subsequently repredicted after ratioing its spectrum against that of the corresponding unspiked oil to

estimate the error contributed by imperfect ratioing of the triglyceride absorptions (as well as to eliminate the possibility that the results of these experiments were confounded by the presence of *trans* isomers in the unspiked oils). The MD between the two sets of *trans* predictions was -0.14, with an SDD of 0.34. These results indicate that the error due to the variability in the underlying triglyceride absorptions among these oils is within the experimental error of the method.

5.5. DISCUSSION

The traditional AOCS method for *trans* analysis by IR spectroscopy has a number of well-known drawbacks, particularly the tendency to significantly overpredict *trans* values at the low end owing to underlying triglyceride absorptions, which is circumvented by converting oils to their methyl esters prior to analysis, and the use of noxious and volatile CS_2 as a solvent. Much effort has gone into overcoming these problems, and several authors have worked on means of eliminating CS_2 (15, 17, 18) by measuring the *trans* content from the IR spectrum of neat oil (or melted fat) or their methyl esters using attenuated total reflectance (ATR) sampling techniques or short-pathlength (~0.1 mm) transmission cells. Although both approaches are workable, each has limitations. The ATR technique is particularly appealing because it requires only that a neat sample be poured onto the surface of the ATR crystal. However, the technique suffers from difficulties in cleaning the ATR crystal (cross contamination) as well as extreme sensitivity to changes in the alignment of the crystal. In the case of short-pathlength transmission cells, the introduction of viscous oil samples into conventional IR cells can be difficult and awkward, and solvent rinses can be problematic in an

industrial setting. In most of the previous work (15, 17, 18) on *trans* analysis of neat oil samples, the critical issue of temperature control has not been addressed, and the sample accessories used were not designed to operate at elevated temperatures and thus were not suitable for the routine analysis of solid fats. Even properly designed heated ATR cells tend to be problematic. Cleaning the crystal surface with solvents changes the surface temperature as a result of evaporative cooling and can lead to solubilization of the epoxy that holds the crystal in place, allowing leakage of solvent and sample to the underside of the crystal, causing gross analytical errors and posing an explosion hazard. To overcome these sampling problems, the McGill IR Group decided to design its own dedicated oil analysis accessory, based on a heated flow-through transmission cell that is designed specifically for the analysis of neat fats and oils (11). The elements of this sealed system, illustrated in Figure 5.1, are a simple three-position valve (off, bypass, and load cell) to facilitate sample handling, a heated cell-bypass line that allows one to rinse the previous sample out of the lines, and an interchangeable transmission flow cell inserted into a stainless steel cell housing. The cell and lines are all maintained at 80 ± 0.2°C, and the sample is prewarmed prior to loading to liquefy it if necessary. Our experience has shown that precise temperature control is essential in obtaining reproducible results, and the mass of the cell housing is crucial in minimizing temperature fluctuations.

Aside from sample handling, the other major limitation in conventional IR *trans* analysis has been the issue of errors caused by underlying triglyceride absorptions. This matter is easily addressed through the use of FTIR spectroscopy by ratioing the single-beam FTIR spectrum of the fat or oil being analyzed against the single-beam spectrum of

a similar reference oil that is free of *trans* double bonds. The ratioing process, and the ability to do it accurately, is one of the advantageous features of FTIR spectroscopy, because it allows one to eliminate the common features associated with any two spectra, producing a "differential spectrum" (19) that accentuates the differences between similar samples. As pointed out by a number of authors (17, 20, 21), ratioing the single-beam spectrum of the sample against that of a *trans*-free oil cancels out the absorption features associated with the triglyceride backbone that underlie the *trans* band and removes the variable baseline tilt caused by the proximity of strong triglyceride fingerprint bands that make it difficult to draw a proper baseline in a conventional absorbance spectrum. The underlying absorptions cause significant overprediction of the *trans* content in low-*trans* samples, while the sloping baseline gives rise to uncertainty in the peak height measurement. With the ratioing approach, the isolated *trans* content can be obtained unambiguously by the AOCS peak height calculation without any need to convert the oil to methyl esters, thus drastically simplifying the method. As shown in Table 5.2 and discussed in detail elsewhere (16), similar results can be obtained with PLS regression, a form of factor analysis, which allows the underlying absorptions and baseline fluctuations to be mathematically modeled within the calibration; however, as noted previously, many analysts are not familiar with this approach.

In making use of the ratio approach, the suitability of a particular reference oil, and hence the range of applicability of the calibration equation developed with that reference oil, is subject to limitations imposed by the variability of the underlying absorptions among different triglycerides. For example, when analyzing partially hydrogenated soybean oils, Mossoba *et al.* (17) found that the *trans* values obtained by

using triolein as the reference oil were 2.6 percentage points higher than those obtained when a refined, bleached, and deodorized soybean oil served as the reference material. They attributed this difference primarily to the more accurate ratioing out of the underlying triglyceride absorptions in the latter case. In the present work, this issue was further investigated by spiking five oils of different types with trielaidin at levels of 1.5-7%. We selected this concentration range as a "worst-case scenario", because any error due to underlying absorptions would be greatest in samples containing low levels of *trans* isomers. As reported above, these spiking experiments indicated that the residual underlying triglyceride absorptions after the ratioing procedure did not contribute a significant error, because the FTIR *trans* predictions obtained were within one percentage point of the gravimetric values for all the spiked samples. This suggests that the application of the ratioing method based on the use of a single reference oil and the corresponding calibration equation may serve as a generalized procedure for *trans* determination in a wide variety of edible fats and oils.

The spectral ratioing approach is a simple means by which to eliminate the need for either solvents or methylation, and these advantages have led to ratioing being proposed for adoption by the AOCS as Recommended Practice Cd 14b-95 for the quantitation of isolated *trans* isomers at levels equal to or greater than 1% (20). We concur with this recommendation but suggest that the methodology be further standardized and automated through the use of a reliable, functional heated transmission cell and programming of the spectrometer. Although most FTIR spectrometers come equipped with basic software that allows for the measurement of peak heights and areas and have simple "quant packages" that allow for construction of Beer's law plots, it can

be cumbersome to carry out the appropriate baseline selection, measure peak heights, and produce the corresponding *trans* values. One of the goals of the McGill IR Group is to take FTIR spectroscopy out of the technical environment and make it a routine quality control tool, independent of specialized expertise. This can readily be done by programming the spectrometer, in this case by using Visual Basic, to produce a user-friendly interface between the operator and the instrument. The operator's sole function is to present the sample to the instrument, and the software takes care of the rest of the analysis and data presentation. This approach has many benefits in the industrial and even the analytical setting, in that there is no major learning curve associated with the analysis, making it possible for plant personnel to carry out *trans* analyses on a routine basis. Accordingly, a software package based on our modified FTIR AOCS protocol has been developed; it simply presents the results in a spreadsheet after each sample has been aspirated into the IR cell. The analysis takes about 2 min per sample.

The software package also implements concepts of calibration transfer, whereby the calibration can be implemented directly from the software package and adjusted to suit the spectrometer and cell pathlength being used, eliminating the requirement to physically carry out a calibration. The program incorporates a calibration derived for a 25-μm KCl cell, which is the optimal pathlength for analysis over the range of 0-100% *trans*. In terms of sensitivity, the optimal pathlength depends on the maximum *trans* value to be measured. For instance, a 50-μm cell provides optimal sensitivity in the 0-50% *trans* range without exceeding the limit of detector linearity. The calibration transfer routine allows the 25-μm calibration to be restandardized for use with longer pathlength

cells through the use of a gold standard and has been demonstrated to be capable of compensating for pathlength differentials up to 100%. This calibration transfer routine also allows the calibration to be implemented on another instrument and compensates for background and instrument drift over time and cell wear. These features are standard in several of our other oil analysis packages and have proven to perform very well.

This study indicates that the basic AOCS *trans* analysis methodology can be updated and simplified dramatically through the use of the ratioing capability of FTIR spectrometers as well as a temperature-controlled sample-handling accessory. The methodology, as packaged and programmed by the McGill IR Group for use with a custom-designed oil analysis accessory, produced results consistent with the mean results obtained for the 1995 Smalley check sample set by a number of laboratories using the traditional AOCS method. The protocol devised eliminates the need for solvents and methylation, and because the calibration equation is part of the software, which can be installed on any FTIR spectrometer operating under Omnic software, the need for calibration can be eliminated. Standardization of the methodology with proper accessories, temperature control, and programming would help to facilitate the routine determination of *trans* content in fats and oils. It is suggested that the McGill IR Group *trans* analysis protocol, which requires a minimum of sample manipulation and operator skill, be considered as a modification of the newly proposed Recommended Practice Cd 14b-95.

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BRIDGE

In Chapter 4, the results of preliminary validation studies of the triglyceride-based PLS calibration models developed for the prediction of IV and *cis* and *trans* content were described. These studies included the analysis of a variety of fats and oils that had been preanalyzed for IV by the standard iodometric method, with the accuracy of the FTIR-predicted *cis* and *trans* contents being evaluated by linear regression of $\Sigma cis + trans$ against chemical IV. However, the individual accuracies of the *cis* and *trans* predictions could not be determined owing to the lack of availability of a convenient, accurate reference method. The development of the *trans* peak height method described in Chapter 5, based on the spectral ratioing principle employed in the FTIR/ATR method adopted as an AOCS Recommended Practice shortly thereafter, furnished a suitable reference method for additional validation of the PLS calibration models discussed in Chapter 4. The following chapter reports the results of an extensive validation study, entailing the comparison of the *trans* values obtained from the PLS-based and *trans* peak height methods for over 100 samples of partially hydrogenated oil provided by an industrial collaborator, as well as comparison with GC data.

CHAPTER 6

VALIDATION OF FTIR *TRANS* AND IODINE VALUE ANALYSES OF FATS AND OILS

6.1. ABSTRACT

An FTIR edible oil analysis package designed to simultaneously analyze for *trans* content, *cis* content, iodine value (IV), and saponification number (SN) of neat fats and oils by using calibrations based on pure triglycerides and derived by application of partial-least-squares (PLS) regression was assessed and validated. More than 100 hydrogenated rapeseed and soybean samples were analyzed by using the edible oil analysis package as well as the newly proposed modification of the AOCS IR *trans* method with trielaidin in a *trans*-free oil as a basis for calibration. In addition, ~1/3 of the samples were subsequently reanalyzed by gas chromatography (GC) for IV and *trans* content. The PLS approach predicted somewhat higher *trans* values than the modified AOCS IR method, which was traced to a combination of the inclusion of trilinolelaidin in the calibration set and the effects of baseline fluctuations. Eliminating trilinolelaidin from the triglyceride standards and the use of second-derivative spectra to remove baseline fluctuations produced excellent concurrence between the PLS and modified AOCS IR methods (mean difference of 0.61% *trans*). Excellent internal consistency was obtained between the IV and *cis* and *trans* data provided by the edible oil analysis package, the relationship being very close to that theoretically expected [IV = 0.86(*cis* + *trans*)]. The IV data calculated for the GC-analyzed samples matched the PLS IV predictions within 1 IV unit. The *trans* results obtained by both IR methods were linearly related to the GC

data; however, as is commonly observed, the GC values were significantly lower than the IR values, the GC and IR data being related by a slope factor of ~0.88, with an SD of ~0.80. The concurrence between the *trans* data obtained by the two FTIR methods and between the FTIR and GC IV data, as well as the internal consistency of the IV, *cis*, and *trans* FTIR predictions, provide strong experimental evidence that the edible oil analytical package measures all three variables accurately.

6.2. INTRODUCTION

Edible fats and oils vary in their triglyceride makeup, relative degree and forms of unsaturation (*cis* or *trans*), weight-average molecular weight, and overall fatty acid composition/distribution, and these complex determinants define the physicochemical properties of the lipid system. For stability and functionality reasons, oils are often hydrogenated and converted into fats. Hydrogenation reduces the overall degree of unsaturation but also leads to increased levels of *trans* fatty acids, now of increasing concern to nutritionists owing to their association with heart disease (1). *Trans* fatty acid levels can reach values of 40% or more in hardened fats, and consideration is being given to legislation to require the labeling of the *trans* content in fat-based products, such as margarines, spreads, and frying fats (2). For oil processors, iodine value (IV) and *trans* content are important process parameters that require monitoring during hydrogenation to ensure that consistent products with desired physical properties are produced. As such, the availability of a simple, rapid, and routine means of determining IV and *trans* content directly on neat fats would be of substantial benefit.

The McGill IR Group has been carrying out research on the development of simple, rapid, and accurate methods of edible oil analysis based on FTIR spectroscopy (3-11). One of the practical developments of this research has been an FTIR edible oil analyzer, which is capable of simultaneously determining IV, saponification number, and *cis* and *trans* content (IV/SN/*cis*/*trans*) in a single analysis on a neat fat or oil in less than 2 min. The system is preprogrammed and precalibrated and has a heated sample-handling accessory (8) to allow both fats and oils to be analyzed in their liquid state at 80°C; a commercial version is being marketed by Nicolet Instrument (Madison, WI). This system is being used by a number of edible oil processors, who are satisfied with its performance; however, it has been noted through our own as well as the users' experience that, although the edible oil analysis package employed by the system yields *trans* and IV predictions that are linearly related to the reference analyses, the *trans* values tend to be overestimated. This is readily corrected for by linear regression; however, because the original concept for the development of the method is to be independent of recalibrations or adjustment, we felt that this problem should be addressed by a structured validation study. This study, carried out in cooperation with a major European edible oil processor, compares the *trans* and IV data obtained from the FTIR edible oil analysis package with the *trans* data obtained from a modification of the standard AOCS IR method (11) as well as *trans* and IV data obtained by GC analyses (12).

6.3. MATERIALS AND METHODS

Analytical System. The instrument used was a Nicolet Magna 510 FTIR spectrometer equipped with a heated sample-handling accessory, manufactured by

Dwight Analytical (Toronto, Ontario, Canada), and a 25-μm KCl flow cell heated to 80°C. The instrument was controlled by a 486 PC running under Nicolet® Omnic 2.1 software and preprogrammed in Microsoft® Visual Basic to carry out IV/SN/*cis*/*trans* analyses by using previously developed calibrations based on pure triglyceride standards and derived by application of the partial-least-squares (PLS) regression technique (3, 8). A calibration equation for the determination of *trans* content from a modified version of the AOCS IR method (Cd 14-61) (12) was derived and appended to the PLS analytical package to produce analytical values concurrently from the same spectra. For calibration of the modified AOCS method (11), five calibration standards (0-50% trielaidin) were prepared by addition of trielaidin (Sigma Chemical Co., St. Louis, MO) to an unhydrogenated soybean oil having a *trans* content of <0.1% (as determined by GC). Calibration was performed by recording the spectra of these standards using the same sample-handling accessory, flow cell, and temperature conditions as described above and ratioing the spectrum of each standard against that of the base oil. The concentrations of trielaidin in these standards were related to the peak heights measured in the differential spectra at 966 cm^{-1}, corresponding to the position of the peak maximum of trielaidin in the spectra of the calibration standards, relative to a baseline drawn between 995 and 937 cm^{-1}. The spectra of all samples analyzed by the modified AOCS method were ratioed against the same base oil spectrum as the calibration standards.

Samples/Analyses. One hundred and eight samples of hydrogenated rapeseed (n = 36) and soybean (n = 71) oils were obtained from Vandemoortele (Izegem, Belgium) for analysis by FTIR spectroscopy. For all FTIR analyses, the samples were prewarmed in a

microwave oven to ~80°C and aspirated through the IR cell. All the samples supplied were analyzed for IV and *cis* and *trans* content by the FTIR edible oil analysis package (6) as well as for *trans* content by the modified AOCS peak height method (11). The data output was stored to a text file in a spreadsheet format for subsequent statistical analysis. Twenty of the samples were run in duplicate to determine repeatability (back-to-back duplicates) and were also reanalyzed four weeks later to assess the between-run precision of the FTIR method. *Cis* and *trans* data were expressed as % triolein and % trielaidin, respectively, and internal analytical consistency was determined by relating total unsaturation [Σ(*cis* + *trans*)] to IV. Approximately one-third of the samples supplied were independently analyzed by P. Maes at the Vandemoortele laboratory in Belgium for *trans* content by the AOCS GC procedure (Method Ce 1c-89) (12), and their IVs were determined from the GC data by the Calculated Iodine Value method (Cd 1c-85) (12). A Hewlett-Packard gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector and capillary injection system, was operated isothermally (192°C) with a 20-m SP-2340 (Supelco Inc., Bellefont, PA) column and the injection port and detector set at 250°C. Helium was used as the carrier gas at a flow rate of 15 cm/s and a column head pressure of 125 kPa. Methyl esters of the oil samples were prepared by the AOCS (Ce 2-66) boron trifluoride method, and ~0.5-1 μL was injected for quantitative analysis; the percentage *trans* content was determined by relating the integrated *trans* fatty acid peak areas to the total area for all fatty acids eluted. The IV and *trans* GC validation data obtained at the .Vandemoortele laboratory were compared to the corresponding FTIR results by linear regression and Z-linear regression (data forced through the origin), as

well as in terms of mean difference (MD) and standard deviation of the differences (SDD) (13).

6.4. RESULTS AND DISCUSSION

6.4.1. Infrared Determination of *trans* Content

The IR spectroscopic determination of the *trans* content of fats and oils is a well-established AOCS method (12). This method is based on the measurement of the peak height of a characteristic absorption band of isolated *trans* bonds at 10.3 μm (970 cm^{-1}). However, the procedure has a number of well-known drawbacks, such as the use of volatile and noxious/toxic CS_2, and suffers from interferences due to overlapping triglyceride absorptions, which cause the *trans* values to be overestimated (14). To circumvent the latter problem, the AOCS method requires that samples be saponified and the fatty acids converted to their methyl esters prior to analysis. In recent years, as a result of the development of FTIR instrumentation and computerized data analysis techniques, various modifications to the AOCS method for the IR determination of *trans* content have been investigated. Several of these (15-17) measure the *trans* content from the IR spectrum of neat oil (or melted fat) or methyl esters recorded in a short-pathlength (~0.1 mm) transmission cell or by the attenuated total reflectance (ATR) technique, thereby eliminating the use of CS_2. Because of the inconvenience of injecting samples into short-pathlength cells and the drawbacks of the ATR technique, particularly the difficulty of cleaning the ATR crystal and the extreme sensitivity of FTIR/ATR measurements to changes in the alignment of the crystal and temperature fluctuations, the McGill IR Group developed a *trans* method based on the use of a heated flow-through transmission cell

sample-handling accessory, designed specifically for the analysis of neat fats and oils (11). Another modification to the AOCS method recently suggested (11, 15, 18, 19) is to remove the underlying triglyceride absorptions by ratioing the single-beam FTIR spectrum of the fat or oil being analyzed against the single-beam spectrum of a similar reference oil that is free of *trans* groups. This approach allows the isolated *trans* content to be obtained unambiguously as per the AOCS peak height calculation and avoids the use of CS_2 as well as the saponification and methylation procedures, leading to a substantial simplification of the method. This procedure, applicable to quantitation of isolated *trans* isomers at levels equal to or greater than 1%, has been proposed for adoption by the AOCS as Recommended Practice Cd 14b-95 (18).

An alternative approach to the problem of underlying absorptions was employed in our previous development of a *trans* method (8). By employing PLS, a form of factor analysis, we were able to develop a calibration model for the prediction of *trans* content that, in principle, accounts for the triglyceride backbone absorptions that contribute to the intensity of the *trans* absorption band. The PLS approach has the additional benefit of allowing the determination of IV and *cis* content in addition to *trans* content, as well as SN, and has been developed as an integrated analytical package. PLS is effectively a "whole spectrum" approach, which relates spectral information from broad regions of the spectrum to the compositional variable of interest, as opposed to being based on a single-frequency measurement. Thus, it is a powerful multivariate analysis technique that is capable of accurately measuring components in the presence of interfering absorptions, while providing greater accuracy than can be achieved with single-frequency measurements (20). The modified AOCS method described above and the PLS approach

both attempt to compensate for overlapping absorptions but in distinctly different ways, and, accordingly, it was of interest to examine their relative concurrence by comparing the predictions obtained from these two methods for a common set of samples.

Figure 6.1A shows the *trans* absorption region (995-937 cm^{-1}) in the overlaid spectra of the standards employed to derive the calibration equation for the modified AOCS method, prepared by adding varying amounts of trielaidin to a *trans*-free base oil. The corresponding spectra obtained by ratioing the single-beam spectra of these calibration standards against the single-beam spectrum of the base oil are presented in Figure 6.1B. The horizontal baseline produced by the ratioing procedure eliminates some of the uncertainty in the measurement of the *trans* peak height. The standard curves derived from the spectra shown in Figures 6.1A and 6.1B are presented in Eqs. [6.1] and [6.2], respectively:

$$\%\ trans = -3.917 + 131.276\ A(966) \qquad R = 0.999 \qquad SE = 0.430 \qquad [6.1]$$

$$\%\ trans = -0.230 + 130.973\ A(966) \qquad R = 0.999 \qquad SE = 0.398 \qquad [6.2]$$

where: % *trans* = *trans* content expressed as % trielaidin

A(966) = absorbance at 966 cm^{-1} relative to a baseline drawn between 995 and 937 cm^{-1}

Equation [6.1] shows that the underlying triglyceride absorption in the spectrum of the *trans*-free oil corresponds to an intercept value of 4% *trans*, whereas the intercept is eliminated from the calibration equation by the ratioing procedure. Equation [6.2] was employed to predict the *trans* contents of 108 samples of hydrogenated rapeseed and

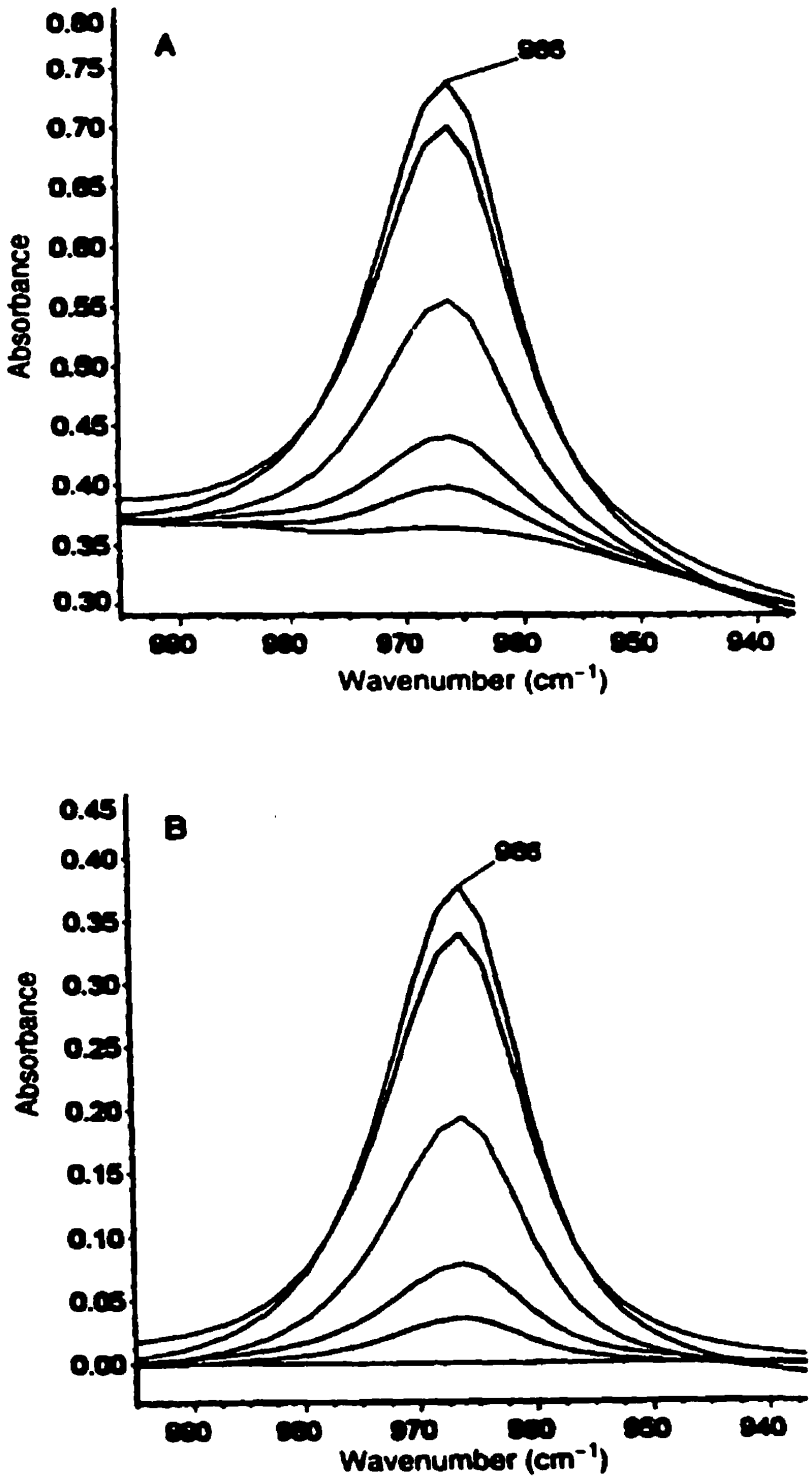

Figure 6.1. (A) Overlaid spectra in the *trans* absorption region of calibration standards prepared by addition of various amounts of trielaidin to a *trans*-free base oil; (B) same spectra as in (A) after ratioing out the spectrum of the base oil.

soybean oils after ratioing their spectra against that of the same base oil used in preparing the calibration standards. The *trans* values of the samples were also predicted from the PLS calibration model incorporated in the FTIR edible oil analysis package. Figure 6.2 presents a comparison of the % *trans* values predicted by the modified AOCS method and the PLS method for the 108 samples. The linear regression equation obtained for Figure 6.2 was:

$$\%\ trans\ (\text{PLS}) = 1.930 + (1.029)\%\ trans\ (\text{AOCS}) \quad SE = 0.692 \quad R = 0.998 \qquad [6.3]$$

This equation indicates that the % *trans* values are overestimated by the PLS method as compared to the modified AOCS method. The mean difference (MD) between the two sets of predictions revealed a bias of 3.05 *trans* units, with a standard deviation of the differences (SDD) of 0.78. Furthermore, examination of the plot in Figure 6.2 indicates that several of the PLS-derived % *trans* values are substantially higher (5-6% *trans*) than the values obtained from the modified AOCS method. Because the predictions being compared are based on a common set of spectra, experimental sources of error, such as instrumental noise or sampling and temperature variations, cannot account for the observed discrepancies. The general overestimation of *trans* content by the PLS method was subsequently confirmed by analyzing the gravimetrically prepared trielaidin standards used to calibrate the modified AOCS method. The results obtained confirmed our hypothesis that the PLS method tends to predict somewhat higher *trans* values and led to a detailed reexamination of the PLS calibration model. We found that by excluding trilinolelaidin (C18:2*tt*) from the calibration set and basing the calibration on second-derivative spectra to decrease the sensitivity of the calibration model to baseline

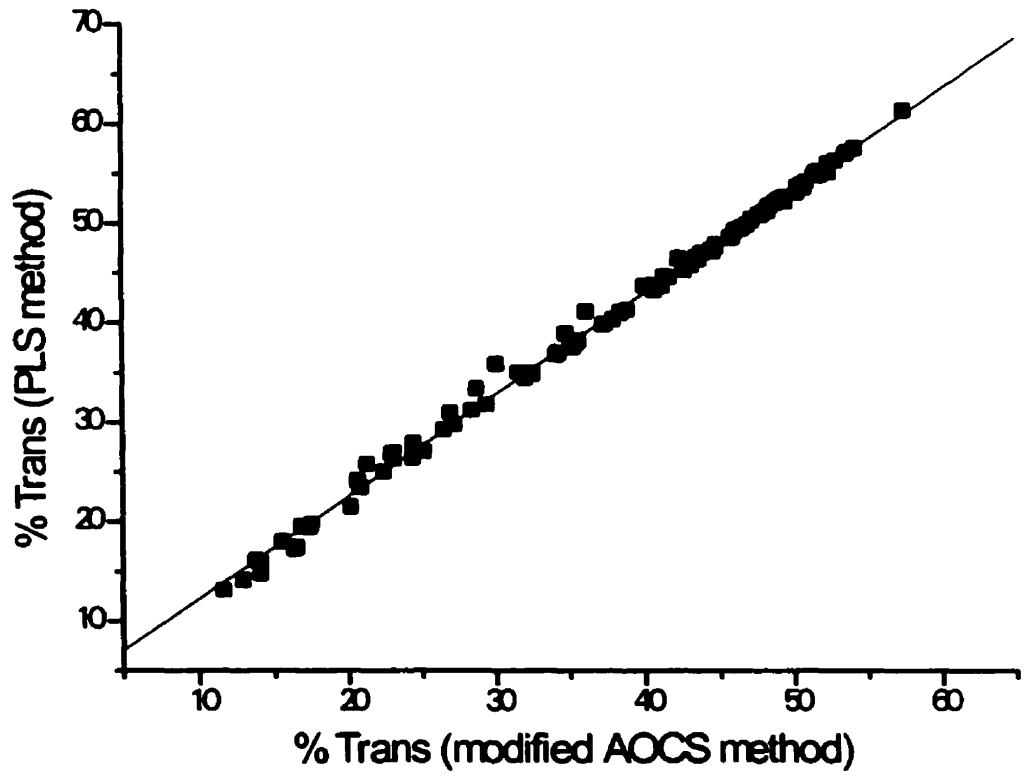

Figure 6.2. Plot of % *trans* values predicted using the PLS calibration for hydrogenated rapeseed and soybean oils vs. the % *trans* values obtained by the modified AOCS method.

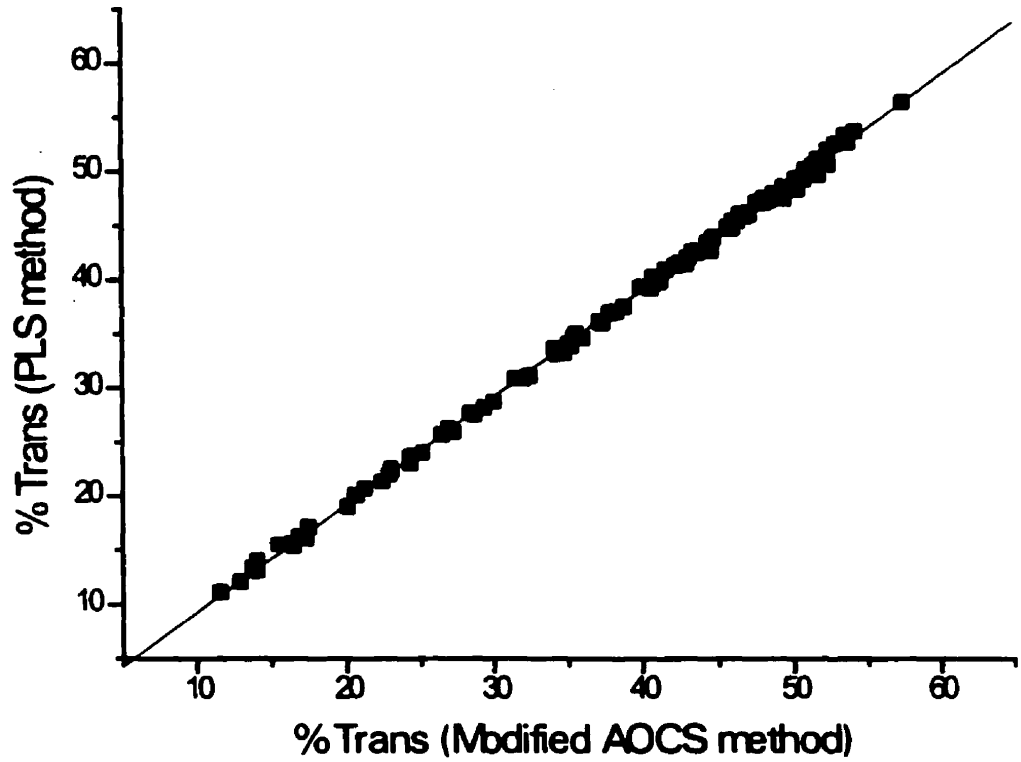

Figure 6.3. Plot of % *trans* values predicted using the refined PLS calibration for hydrogenated rapeseed and soybean oils vs. the % *trans* values obtained by the modified AOCS method.

fluctuations, the PLS predictions could be reconciled with both the known concentrations of the gravimetrically prepared standards and the modified AOCS predictions for the samples; the latter results are shown in Figure 6.3, yielding the following linear regression equation:

$$\%\ trans\ (\text{PLS}) = -0.151 + (0.988)\%\ trans\ (\text{AOCS}) \quad SE = 0.353 \quad R = 0.999 \qquad [6.4]$$

Comparison of Eqs. [6.3] and [6.4] and the corresponding SEs reveals that the refined PLS calibration model provides much better concurrence with the modified AOCS method, as also demonstrated by reduction of the MD between the two sets of predicted values to -0.61 *trans* units. In addition, the fact that good agreement could be obtained between the PLS and modified AOCS methods indicates that both methods successfully compensate for the overlapping absorptions that cause interferences in the traditional AOCS method.

6.4.2. Internal Consistency of the Data

A key element in assessing the FTIR-PLS approach is to ensure that the *cis* and *trans* data are internally consistent with the IV data, because the two measures are related. Because the *cis* and *trans* data provided by the FTIR edible oil analysis package are expressed in terms of % triolein and % trielaidin, the IV is related to the sum of the *cis* and *trans* double-bond contributions in the following manner:

$$IV = 3[(C + T)\ (\text{Mw } I_2/\text{Mw TG})] \qquad [6.5]$$

$$= 0.8601(C + T) \qquad [6.6]$$

where:
- C = % *cis* (as triolein)
- T = % *trans* (as trielaidin)
- Mw TG = Molecular weight of triolein or trielaidin (885.40)
- Mw I_2 = Molecular weight of molecular iodine (253.81)

Based on this relationship, one would expect the FTIR-predicted Σ*cis* + *trans* to be linearly related to the FTIR-predicted IV, and a plot of 0.8601(Σ*cis* + *trans*) versus IV to produce a slope of 1.0 and an intercept of 0.0. Figure 6.4 presents a plot of FTIR-predicted IV for the 108 rapeseed and soybean samples versus the sum of predicted *cis* and predicted *trans* multiplied by the theoretical slope factor of 0.8601. The linear regression equation obtained for Figure 6.4 was:

$$IV = 0.0119 + 1.0002\ CT \qquad SE = 0.069 \qquad R = 1.0000 \qquad [6.7]$$

where: CT= 0.8601(Σ*cis* + *trans*). Elimination of the intercept by means of a regression that forces the data through the origin produced a slope of 0.998, within 0.2% of the theoretical slope of unity. Multiple regression (forced through the origin) of the IV data versus the % *cis* and % *trans* data produced coefficients of 0.858 and 0.859, respectively, very close to the ideal value of 0.8601 expected from Eq. [6.6]. These results provide strong evidence that the IV and *cis*/*trans* predictions obtained with the FTIR analytical package are internally consistent, in that they reflect the theoretical interrelationships one expects to observe among the three parameters. Furthermore, the relationship between the IV and *cis*/*trans* predictions was consistent for both rapeseed and soy samples, demonstrating that the FTIR-PLS results are oil-independent.

6.4.3. Repeatability and Between-Run Precision of the FTIR Data

The repeatability of the FTIR data was assessed by comparing the IV, SN, *cis*, and *trans* predictions obtained from the edible oil analysis package for 20 samples from duplicate spectra of each sample, recorded consecutively. The results for each of the four measures are presented in Table 6.1, in terms of the mean difference for repeatability

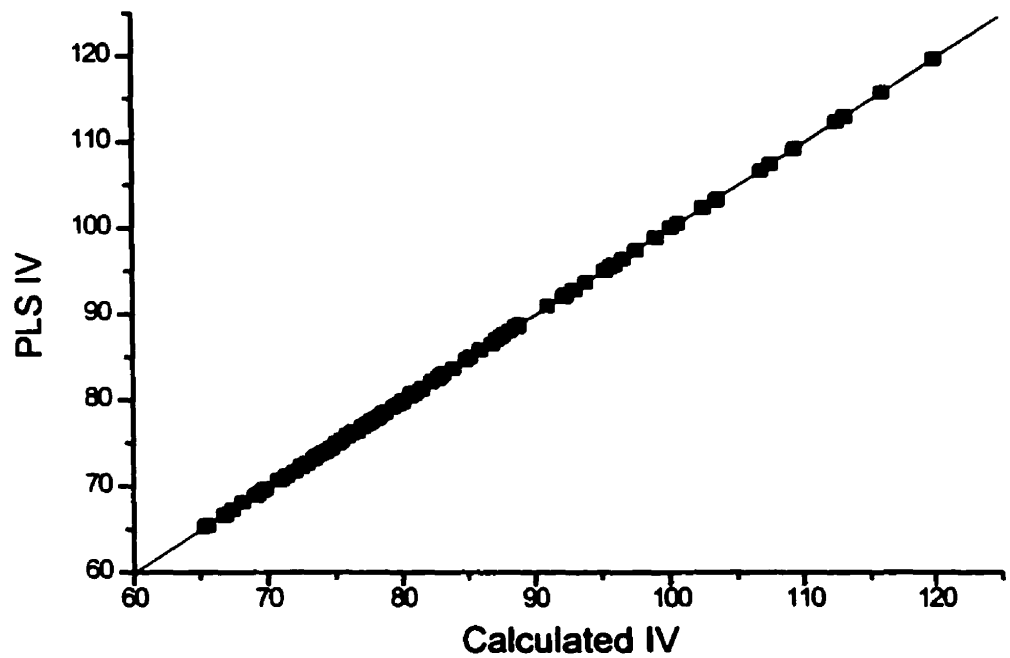

Figure 6.4. Plot of FTIR-PLS predicted IV for hydrogenated rapeseed and soybean oils vs. the IV calculated using the FTIR-PLS predicted *cis* and *trans* values multiplied by the slope factor of 0.8601, determined from Eq. [6.5].

(MD_r) and the standard deviation of the differences for repeatability (SDD_r), the standard deviation obtained from linear regression of the duplicate predictions, and the corresponding coefficient of variation (CV). The repeatability data indicate no significant biases between duplicates, the ideal value for the MD_r being zero. The SDD_r, which represents the variability around the mean difference, is quite similar to the SD obtained by linear regression. The CV, which expresses overall variability in percent terms relative to the overall mean value of the samples, is less than 0.3% for all components.

After 4 weeks, over which the instrument was configured for other analyses which required removal of the cell, the FTIR system was restandardized and 20 randomly selected samples were run to determine the between-run precision. Table 6.2 presents the MD and SDD calculated by comparing the predictions originally obtained to those obtained 4 weeks later. These results indicate that there is no bias *per se* in the data; however, as expected, there is a small increase in the variability around the MD, reflected by a general increase in the CV. However, the between-run precision is well within 1% for all the parameters measured.

6.4.4. Validation Data

Of the global set of samples analyzed by FTIR spectroscopy, about one-third were selected for validation analysis by capillary GC (IV and *trans*). The GC data obtained are compared in Table 6.3 to the corresponding FTIR-predicted values for these samples (i.e., IV and % *trans* from the PLS-based FTIR edible oil analysis package and % *trans* from the modified AOCS method).

Table 6.1. FTIR Predictions for IV, SN, and *cis* and *trans* Content: Repeatability for Duplicate Samples (n = 20) Run Consecutively

Measure	IV	SN	*% cis*	*% trans*
Mean	87.74	193.18	61.7	40.15
MD_r	0.048	0.012	0.068	-0.011
SDD_r	0.108	0.109	0.140	0.058
Linear regression SD	0.110	0.104	0.139	0.056
CV	0.13%	0.05%	0.23%	0.14%

Table 6.2. FTIR Predictions for IV, SN, and *cis* and *trans* Content: Between-Run Precision for Samples Run Four Weeks Apart

Measure	IV	SN	*% cis*	*% trans*
MD	-0.179	0.437	-0.057	-0.074
SDD	0.502	0.269	0.484	0.221
CV	0.57%	0.14%	0.78%	0.55%

Table 6.3. Validation *trans* and IV Data Compared to the Corresponding FTIR Results for 31 Randomly Selected Samples

Sample	GC		FTIR		
	GC IV	GC *trans*	PLS IV	PLS *trans*	AOCS *trans*[a]
R2	80.4	38.06	79.76	42.70	43.34
R3	92.2	20.73	92.06	22.58	22.73
R6	86.0	27.83	84.62	30.97	31.40
R8	100.3	13.06	100.63	13.43	13.54
R10	81.4	41.44	80.74	45.60	45.97
R11	89.5	21.61	88.58	23.73	24.04
R12	89.9	22.03	88.53	23.32	24.04
R13	74.1	48.02	72.27	53.58	53.32
R14	91.2	20.79	81.02	22.38	22.86
R20	98.7	14.13	98.99	15.53	15.25
R28	92.2	18.20	92.32	20.13	20.37
R31	75.8	45.64	76.27	50.72	51.35
R33	96.6	15.56	97.50	17.16	17.09
R34	78.4	45.05	78.14	49.50	50.17
S2	113.9	11.15	115.93	12.15	12.49
S5	96.8	25.49	95.86	27.61	28.11
S6	117.4	10.40	119.89	11.15	11.18
S8	95.4	21.95	96.51	24.06	24.70
S19	65.5	40.16	65.41	47.22	47.67
S25	94.4	21.29	95.11	23.08	24.04
S27	82.2	29.22	80.87	33.20	34.02
S29	103.5	19.99	103.43	21.99	22.21
S35	79.6	36.60	79.50	41.57	42.55
S36	85.1	27.41	84.72	30.95	31.66
S44	72.6	42.84	72.32	48.73	49.38
S47	68.7	39.80	68.14	46.03	46.76
S50	66.8	41.31	66.78	47.68	48.07
S57	76.5	37.32	76.92	42.75	43.47
S59	89.7	28.35	88.79	31.19	32.05
S68	87.5	31.49	87.09	34.71	35.33
S71	111	15.14	112.50	16.24	16.30
MD[b]	-	-	0.004	-3.21	-3.66

[a]Modified AOCS method. [b]Mean difference with respect to the corresponding GC data.

Figures 6.5 and 6.6 present plots of the FTIR-PLS IV versus the GC IV data and the FTIR-PLS *trans* versus the GC *trans* data, respectively. Table 6.4 presents a summary of the linear and Z-linear regression equations derived from these plots and the associated statistics as well as equations that relate the modified AOCS IR *trans* data to the GC *trans* data.

The conventional linear regression equation for IV given in Table 6.4 indicates that there is a combined bias and slope contribution relating the two variables; however, the Z-regression results demonstrate that, in fact, the agreement is excellent because the intercept can be incorporated into the slope without any appreciable increase in the regression error. This is confirmed by the MD of 0.004 IV between the two sets of data. On the other hand, the GC *trans* data, when related to the FTIR *trans* data, whether obtained by the PLS or the modified AOCS method, produced a slope factor of 0.88 rather than a 1:1 correspondence. Accordingly, there is an increasing discrepancy between the IR and GC data as the *trans* content of the samples increases. Similar discrepancies between IR and GC *trans* determinations have been observed by others (21) and have been attributed to unsatisfactory separation of 18:1*t* and 18:1*c* isomers on the currently available GC stationary phases. Given the relative complexity and well-known limitations associated with the GC method, the fact that independently obtained IR and GC *trans* data do track each other well provides additional, indirect evidence for the efficacy of the IR *trans* approaches.

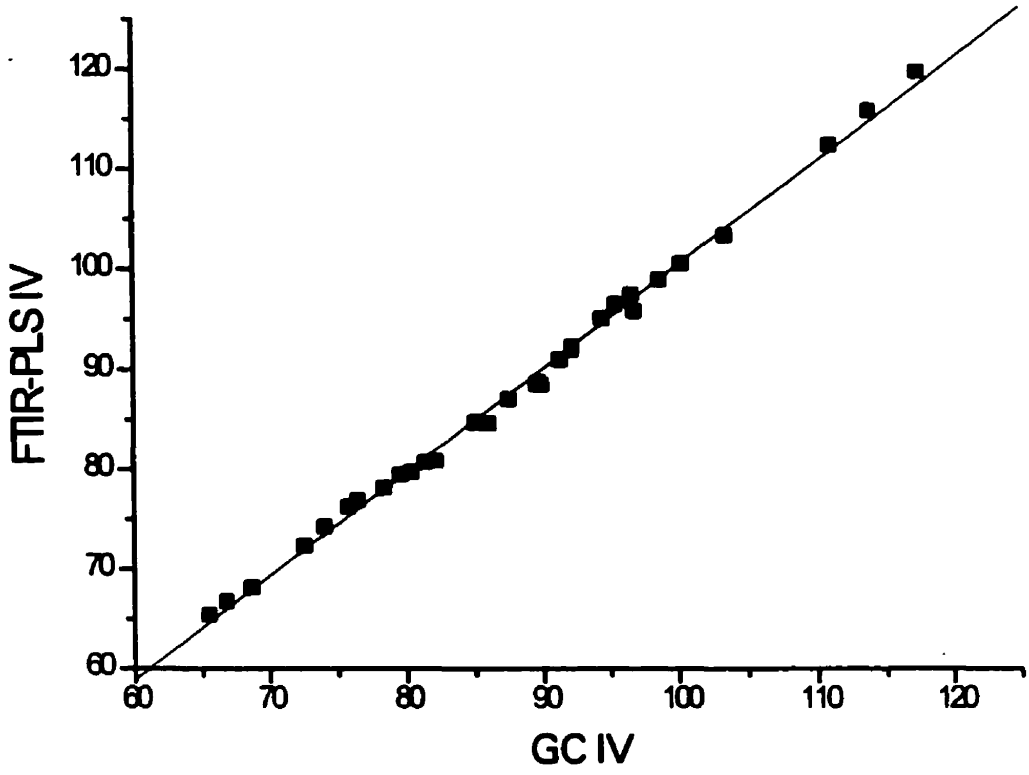

Figure 6.5. Plot of FTIR-PLS predicted IV for 31 hydrogenated rapeseed and soybean oils vs. IV calculated from GC data.

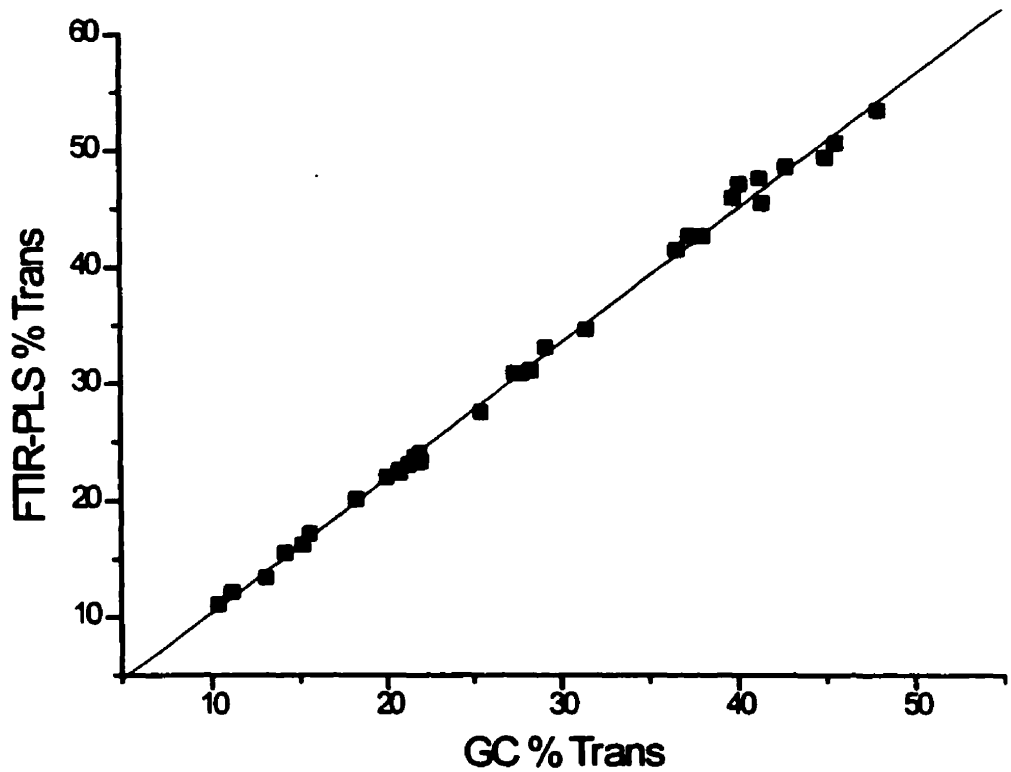

Figure 6.6. Plot of FTIR-PLS predicted % *trans* for 31 hydrogenated rapeseed and soybean oils vs. % *trans* obtained by GC.

Table 6.4. Regression Analysis Summary for Figures 6.5 and 6.6 Plus the Regression of the Modified AOCS IR *trans* data vs. the GC *trans* Data

Regression	Abscissa	Ordinate	Intercept	Slope	SD	R
Normal	GC IV	PLS IV	-3.405	1.0386	±0.783	0.998
Z-Regression	GC IV	PLS IV	0.0000	1.0008	±0.921	0.999
Normal	GC *trans*	PLS *trans*	-1.146	1.1552	±0.736	0.998
Z-Regression	GC *trans*	PLS *trans*	0.0000	1.1199	±0.843	0.999
Normal	GC *trans*	AOCS *trans*	-1.019	1.1663	±0.805	0.998
Z-Regression	GC *trans*	AOCS *trans*	0.0000	1.1350	±0.880	0.999

6.5. CONCLUSION

The initial results obtained in this validation study confirmed that the *trans* predictions obtained from the edible oil analysis package were somewhat higher than those produced by peak height-based IR methods. Refinement of the PLS calibration model corrected this problem and produced good agreement with the results obtained from a modified AOCS method assessed concurrently, the latter having the benefit of employing a small set of calibration standards prepared by a simple and accurate gravimetric procedure. This agreement between the two methods indicates that both methods allow for the accurate determination of *trans* content from neat fats and oils without the need for their conversion to methyl esters, with the PLS method having the advantage that it does not require the availability of a *trans*-free reference oil. In addition, the PLS method provides IV results that match GC IV data, as well as *cis* and SN data. The agreement between total unsaturation (Σ*cis* + *trans*) obtained by GC and that obtained by the FTIR method lends credence to the efficacy of the GC procedure in general, its limitation being the inaccurate partitioning of the *cis* and *trans* components owing to peak overlap. The concurrence of the *trans* and IV data obtained from the PLS-based edible oil analysis package with the *trans* values obtained from the modified AOCS method and with GC IV data, as well as the internal consistency of its IV, *cis*, and *trans* predictions, provide strong experimental evidence that it measures all three variables accurately. This precalibrated and preprogrammed analytical package, carrying out four separate analyses simultaneously (IV/SN/*trans*/*cis*) in a total analysis time of less than 2 min, should be useful for general quality control use in the edible oil industry.

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BRIDGE

In the following chapter, the principles of the triglyceride-based universal PLS calibration models described in Chapter 4 were adopted in the development of a method for the simultaneous determination of IV and *trans* content employing the SB-HATR sample-handling technique. This work was undertaken to expand the applicability of this sample-handling technique in the analysis of edible fats and oils beyond its use in AOCS Recommended Practice Cd 14d-96 for the determination of isolated *trans* isomers by FTIR spectroscopy, which was approved as an AOCS Official Method in 1999.

CHAPTER 7

SIMULTANEOUS DETERMINATION OF IODINE VALUE AND *TRANS* CONTENT OF FATS AND OILS BY FTIR SPECTROSCOPY EMPLOYING AN SB-HATR ACCESSORY

7.1. ABSTRACT

A method for the simultaneous determination of iodine value (IV) and *trans* content from the FTIR spectra of neat fats and oils recorded with the use of a heated single-bounce horizontal attenuated total reflectance (SB-HATR) sampling accessory was developed. Partial-least-squares (PLS) regression was employed for the development of the calibration models, and a set of 9 pure triglycerides served as the calibration standards. Regression of the FTIR/PLS-predicted IVs and *trans* contents for 10 partially hydrogenated oil samples against reference values obtained by gas chromatography yielded slopes close to unity and standard deviations (SDs) of <1. Good agreement (SD < 0.35) was also obtained between the *trans* predictions from the PLS calibration model and *trans* determinations performed by the recently adopted AOCS FTIR/SB-HATR method for the determination of isolated *trans* isomers in fats and oils.

7.2. INTRODUCTION

Infrared (IR) analysis of fats and oils has traditionally involved dissolution of the sample in a suitable solvent, such as CS_2 or CCl_4, to allow the sample to be injected into a standard transmission IR cell. In recent years, several sample-handling approaches that allow IR spectra to be recorded directly from fats and oils in their neat form have been explored. Our group designed a heated transmission flow cell accessory that meets the

requirements for rapid, semiautomated analysis of fats and oils (1) and has developed a number of analytical methods that are based on the use of this accessory (2-6). Horizontal attenuated total reflectance (HATR) accessories have also been widely used in the development of FTIR methods for the analysis of fats and oils (7-13) because they provide a simple and convenient means of sample handling (14). Oils are simply pipetted onto the surface of an ATR crystal, and premelted fats can be handled analogously provided that the crystal is maintained at a temperature above the melting point of the fat. When only small amounts of sample are available, the single-bounce (SB) HATR accessories that have become available in recent years are particularly useful owing to the small volume of sample required (<50 μL) to cover the surface of the ATR crystal.

Recently, the AOCS adopted a new IR method for the determination of isolated *trans* isomers in fats and oils that uses an SB-HATR accessory (15). Mossoba and co-workers have described the principles of this method (13), its advantages over the traditional AOCS IR *trans* method, and its potential applicability to the analysis of fats extracted from foods (16). The adoption of this simple and rapid method by the AOCS is timely in view of the U.S. Food and Drug Administration's recently proposed amendment of the regulations on nutrition labeling, which requires that the amount of *trans* fatty acids present in a food be both included in the amount declared for saturated fatty acids and stated in a footnote at the bottom of the nutrition label. Although the principles of the new AOCS IR method can be implemented by using a transmission cell (6), it may be anticipated that most laboratories adopting this method will acquire SB-HATR accessories, since this sample-handling approach is specified in the official method. Accordingly, we felt that it would be of interest to evaluate the broader applicability of

the SB-HATR sample-handling technique for the bulk characterization of fats and oils by FTIR spectroscopy. In our previous work with a heated transmission flow cell accessory, we developed an edible oil analysis package capable of simultaneously determining iodine value (IV), saponification number, and *cis* and *trans* content in a single analysis on a neat fat or oil in less than two minutes (2). The objective of the present work was to develop an SB-HATR method for the simultaneous determination of IV and *trans* content based on the principles established in this earlier study.

7.3. EXPERIMENTAL

Instrumentation. The instrument employed for this work was a Bio-Rad Excalibur FTIR spectrometer operating under Merlin software (Bio-Rad Laboratories Inc., Cambridge, MA). The spectrometer was equipped with a temperature-controlled SB-HATR sampling accessory (Graseby Specac Inc., Smyrna, GA). The ATR crystal employed was ZnSe, and the temperature of the crystal was maintained at 65°C to allow for the analysis of fats in their liquid state. Both the spectrometer and the optical unit of the sample-handling accessory were purged with a continuous flow of dry air from a Balston dryer (Balston, Lexington, MA) to minimize spectral interferences from water vapor and CO_2.

Development of PLS Calibration Models for the Prediction of IV and trans Content. The calibration set consisted of 9 pure triglycerides (C12:0, C14:0, C16:0, C16:1*c*, C18:0, C18:1*c*, C18:1*t*, C18:2*cc*, and C18:3*ccc*, all obtained from Sigma, St. Louis, MO). Each standard was transferred to two 1-mL vials, equilibrated to 65°C in a heated holding block prior to application of the standard onto the surface of the ATR

crystal. Spectra were collected by co-addition of 512 scans at a resolution of 4 cm^{-1} and were ratioed against a spectrum recorded from the bare ATR crystal immediately prior to acquisition of the spectrum of the standard. The ATR crystal was cleaned with isooctane after collection of each spectrum and allowed to reequilibrate to 65°C prior to application of the next standard.

Calibration models for the prediction of IV and *trans* content were developed using partial-least-squares (PLS) regression (TQAnalyst, Nicolet Instrument, Madison, WI). Duplicate spectra of each calibration standard were employed in the calibration models. The reference values for the standards were determined on the basis of their chemical structure, with *trans* content being expressed as percent trielaidin. The selection of the spectral regions and numbers of factors to be included in the PLS calibration models was based on minimization of the predicted residual error sum of squares (PRESS) in leave-one-out cross-validation (17).

Calibration: trans Content by the AOCS SB-HATR Method. A calibration equation relating *trans* content to the area of the characteristic *trans* absorption band (990-945 cm^{-1}) was derived in accordance with the AOCS SB-HATR method (15). The calibration standards, consisting of triolein/trielaidin mixtures, and the reference oil (a refined, deodorized, and bleached soybean oil) were provided to us as participants in a collaborative study of the method.

Validation. The validation set consisted of 10 samples provided by an oil processor and comprised 2 samples of each of 5 different oil blends. These samples had all been preanalyzed by gas chromatography (18). For the FTIR analysis, samples were warmed in a microwave oven for 2 min and applied as neat liquids onto the surface of the

ATR crystal. Approximately 10 μL of the melted sample was required to cover the surface of the crystal. Single-beam spectra were collected by co-addition of 64 scans at a resolution of 4 cm^{-1}. Cleaning of the ATR crystal between samples entailed wiping off the sample with a lint-free tissue and applying a drop of the next sample and wiping it off.

Two sets of absorbance spectra were generated from the single-beam spectra of the validation samples. One set was obtained by ratioing the single-beam spectra against a background spectrum recorded from the bare ATR crystal prior to collection of the spectra of the validation set (Figure 7.1a) and was employed to predict the IVs and *trans* contents of the validation samples from the PLS calibration models developed in this work. The second set was obtained by ratioing the same single-beam spectra against the single-beam spectrum of a *trans*-free reference oil (Figure 7.1b) and was employed to determine the *trans* contents of the validation samples by the AOCS SB-HATR method. The between-run precision of the FTIR methods was assessed in terms of mean differences (MD_r) and standard deviations of the differences (SDD_r) for duplicate analyses conducted one week apart. To evaluate accuracy, the FTIR-predicted values for IV and *trans* content were regressed against the GC values provided to us by the processor supplying the validation samples.

7.4. RESULTS AND DISCUSSION

The primary objective of the present study was to evaluate the suitability of an SB-HATR accessory as a means of sample handling for the determination of IV by FTIR spectroscopy. As in our work on the FTIR determination of IV with the use of a

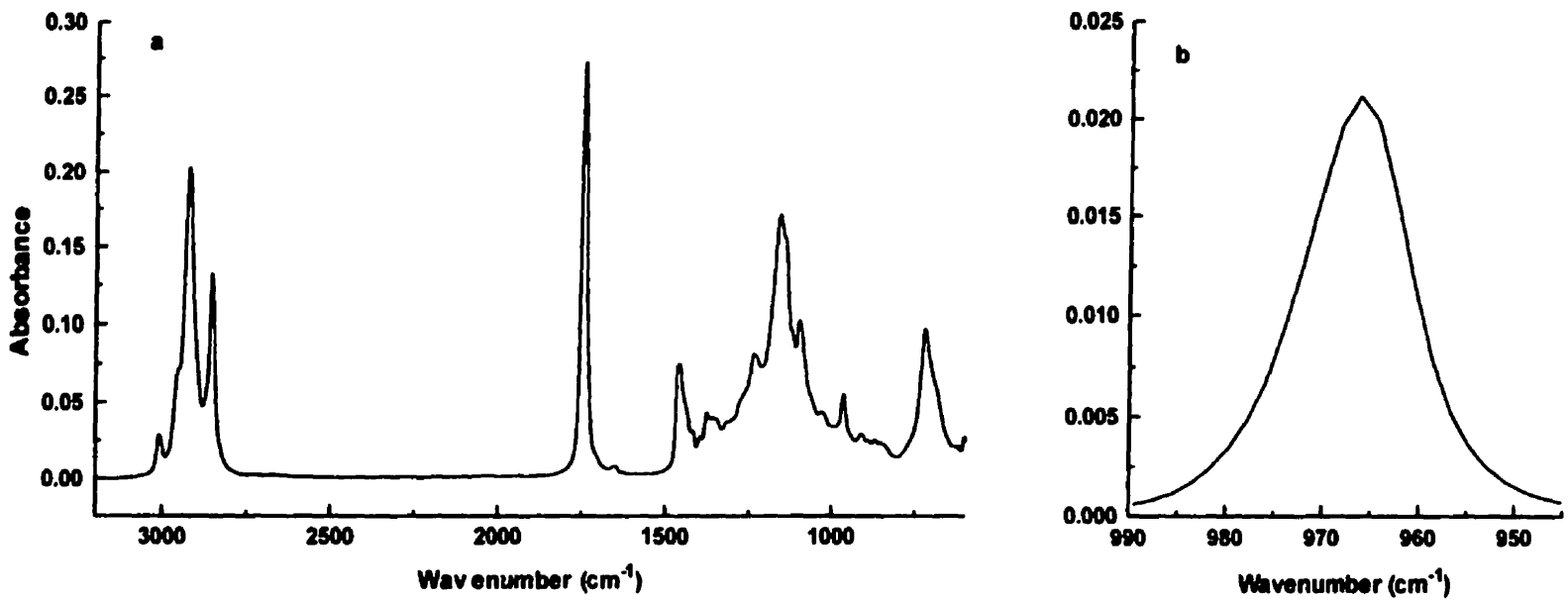

Figure 7.1. (a) FTIR spectrum of a partially hydrogenated vegetable oil recorded with the use of an SB-HATR sampling accessory and ratioed against a background spectrum recorded from the bare ATR crystal: (b) *trans* absorption band in the spectrum of the same sample ratioed against the single-beam spectrum of a *trans*-free reference oil.

transmission flow cell (2) or a multiple-reflection ATR accessory (7), the IV calibration was devised using pure triglycerides as calibration standards and PLS regression. Extensive validation of the calibrations developed in the previous work has demonstrated that this calibration approach yields "universal" calibrations that are applicable to all triglyceride-based oils (7, 19). In addition, the use of a calibration set composed of pure triglycerides has the benefit of eliminating the need for chemical analyses of the calibration standards, as the reference values for the standards are known from their molecular structure, and thus the accuracy of the IR method is not limited by the precision of a reference chemical method. Two spectral regions were found to be optimal in the development of the PLS calibration model for the SB-HATR IV method: 3100-2945 cm^{-1}, which includes the CH stretching absorptions of *cis* and *trans* double bonds, and 2880-2780 cm^{-1}, which encompasses the symmetric CH_2 stretching absorption. Both spectral regions were referenced to a single baseline point at 3200 cm^{-1}. The same set of calibration standards was employed to develop a PLS calibration model for the prediction of *trans* content. The optimized *trans* calibration utilized a single broad spectral region (1110-603 cm^{-1}, referenced to a single baseline point at 1550 cm^{-1}) that includes the *trans*-C=C–H bending absorption at 966 cm^{-1} traditionally employed in IR *trans* determinations. The standard errors of calibration were 0.43 IV and 0.15 % *trans*, with the inclusion of 6 and 5 loading spectra, respectively, in the PLS calibration models.

For validation of the IV and *trans* calibrations, as well as comparison between the results obtained by the PLS and AOCS *trans* methods, a validation set consisting of two samples of each of five different oil blends was employed. These samples had been preanalyzed by the optimized GC method recently adopted by the AOCS (18) and

spanned a wide range of IV (67.8–133.8) and *trans* content (0.3–39.3%). FTIR analyses of these samples were performed in duplicate, one week apart, and the MD_r and SDD_r are summarized in Table 7.1. The corresponding data obtained by analyzing the same set of validation samples using the previously developed transmission-flow-cell method are presented in Table 7.1 for purposes of comparison. The week-to-week variability of the SB-HATR and transmission methods is seen to be comparable, with the values of MD_r for all parameters being close to zero. The results in Table 7.1 also indicate that the between-run precision of the PLS-based *trans* analysis is very similar to that of the AOCS SB-HATR *trans* method.

The correspondence between the FTIR-predicted values of IV and % *trans* and the GC data provided for the validation samples was examined by simple linear regression analysis. Table 7.2 presents a summary of the linear and Z-linear (forced through the origin) regression coefficients and the associated statistics. In all cases, the slopes of the regression equations are close to unity, and the correlation coefficients and regression errors demonstrate an excellent correspondence between the FTIR and GC data. Eliminating the intercept in each of these regression equations by Z-linear regression did not result in an appreciable increase in the regression error, indicating that the FTIR predictions are not biased relative to the GC data.

The results from linear and Z-linear regression of the *trans* predictions from the PLS calibration model against the *trans* data obtained by the AOCS SB-HATR method are also presented in Table 7.2 and show excellent agreement between the two FTIR methods. The traditional IR method for the determination of *trans* isomers, which is based on measurement of the area of the 966-cm^{-1} *trans* absorption band. (20), requires

Table 7.1. Comparison of Week-to-Week Reproducibility of SB-HATR and Transmission-Flow-Cell Methods for the Determination of IV and *trans* Content[a]

Method	IV		% *trans*	
	MD_r	SDD_r	MD_r	SDD_r
SB-HATR (PLS based)	0.42	0.76	-0.19	0.23
Transmission (PLS based)	-0.64	0.51	0.0	0.47
AOCS SB-HATR	NA	NA	-0.16	0.20

[a]MD_r, Mean difference for reproducibility; SDD_r, standard deviation of the differences for reproducibility; $n = 10$.

Table 7.2. Regression Analysis Summary for Figures 7.1-7.3 Plus the Regression of the AOCS SB-HATR *trans* Data versus the GC *trans* Data

Regression	Abscissa	Ordinate	Intercept	Slope	SD	R
Normal	GC IV	PLS IV	-1.33	1.01	0.67	0.999
Z-Regression	GC IV	PLS IV	0.00	0.99	0.73	0.999
Normal	GC *trans*	PLS *trans*	0.42	0.97	0.91	0.998
Z-Regression	GC *trans*	PLS *trans*	0.00	0.98	0.90	0.998
Normal	AOCS *trans*	PLS *trans*	-0.26	0.96	0.29	0.999
Z-Regression	AOCS *trans*	PLS *trans*	0.00	0.96	0.32	0.999
Normal	GC *trans*	AOCS *trans*	0.70	1.00	0.84	0.998
Z-Regression	GC *trans*	AOCS *trans*	0.00	1.03	0.92	0.999

that samples be converted to methyl esters because of interference from underlying triglyceride absorptions. The spectral ratioing capabilities of FTIR spectrometers provide a means of eliminating the need for this time-consuming sample preparation step, because the interfering absorptions can be removed from the absorbance spectrum of the sample by ratioing the single-beam spectrum of the sample against that of a *trans*-free reference oil of similar triglyceride composition. This principle is employed in the AOCS SB-HATR method, whereas the PLS-based *trans* method mathematically compensates for underlying triglyceride absorptions within the calibration model. The excellent correspondence between the results from the two methods provides indirect experimental evidence for the validity of the two different approaches employed to compensate for underlying absorptions. This conclusion is corroborated by the similar good agreement between PLS-predicted *trans* contents and the values from a peak-height calibration based on a spectral ratioing approach that was obtained in earlier work with a transmission flow cell (19).

A noteworthy difference between the results of the latter study and the present work is the improved agreement between the FTIR and GC *trans* data, which we attribute to the use of AOCS Official Method Ce 1f-96 to obtain the GC data for the samples analyzed in the present study. In our earlier work, Z-linear regression of the FTIR data against GC data obtained with AOCS Official Method Ce 1c-89 yielded slopes of 1.12-1.13 (19). In contrast, the Z-regression slopes relating FTIR *trans* to the GC *trans* values obtained with Method Ce 1f-96 are close to unity (0.96 for the PLS method and 1.03 for the AOCS SB-HATR method). Method Ce 1f-96 is optimized to quantify *trans* fatty acids (18), whereas the levels of *trans* isomers in partially hydrogenated oils are

underestimated by Ce 1c-89 owing to incomplete separation of the peaks due to 18:1*c* and 18:1*t* isomers (21). Adam *et al.* (22) compared *trans* data from Method Ce 1c-89 and the AOCS SB-HATR method by analyzing three accuracy standards, prepared by gravimetric addition of trielaidin to a *trans*-free oil, and three partially hydrogenated vegetable oils. Based on the good agreement between the FTIR and GC data, which was peer-verified in an independent laboratory, they concluded that the optimized GC procedure provides accurate *trans* values. The similar results obtained in the present study for a larger number of partially hydrogenated oil samples support this conclusion.

The results of the present study indicate that SB-HATR is a suitable sample-handling technique for the FTIR determination of not only *trans* content, as reported by other workers (13, 22, 23), but also IV. It is noteworthy that the SB-HATR method developed in this work is based on a multivariate calibration approach, as opposed to the simple univariate calibration employed to predict *trans* content in the earlier work. Because minor spectral perturbations can have a major effect on the performance of multivariate calibrations (24), since the calibration models are based on broad spectral regions rather than a single peak, the use of a multivariate calibration approach imposes more stringent requirements on spectral reproducibility than a univariate calibration. For this reason, we had anticipated that the SB-HATR sampling technique might be problematic owing to its short effective pathlength as well as certain limitations of the ATR technique that we had noted in working with a multiple-reflection HATR accessory. In particular, ATR spectra are highly sensitive to temperature variations because the effective pathlength is affected by changes in the refractive index of the sample or the ATR crystal in a wavelength-dependent manner (14). However, by comparison with a

multiple-reflection ATR device, the SB-HATR accessory proved to be more amenable to precise temperature control owing to the much smaller surface area of the ATR crystal. In addition, the temperature fluctuations that result from evaporative cooling during cleaning with solvent were avoided in the present work by adopting the cleaning procedure described in the AOCS SB-HATR *trans* method, whereby the ATR crystal is cleaned by wiping it with a drop of the next sample. The good between-run precision and accuracy obtained in this study provides evidence that this simple procedure is sufficient to avoid significant cross-contamination. We noted that there was a tendency for a film to build up on the heated ATR crystal over time, presumably due to the formation of oxidation products, but this film was readily removed by cleaning the crystal with distilled water. In this regard, it is important to note that the ATR crystal should be scrupulously cleaned with an appropriate solvent (e.g., isooctane) and water prior to recording a background spectrum from the bare crystal, because any residual sample or contaminants on the surface of the ATR crystal will make a substantial contribution to the background spectrum. Based on our experience, we recommend that a background spectrum be collected daily prior to the analysis of samples and after every 20 analyses.

The results of this study indicate that FTIR analysis of neat fats and oils to determine their overall degree of unsaturation as well as *trans* unsaturation can be achieved with the use of either a heated SB-HATR accessory or a heated transmission-flow-cell accessory. The SB-HATR sample-handling technique is simple and convenient, as it requires only that the sample be poured or spread on the surface of the ATR crystal, and is particularly advantageous when the amount of sample is limited, the sample

volume required being <50 μL. As suggested in very early work (25), ATR may also be useful in the monitoring of hydrogenation processes whereas the presence of catalyst particles in oil samples can be problematic in transmission-flow-cell measurements owing to possible clogging of the cell and light scattering effects. On the other hand, the limited sensitivity of the SB-HATR technique, due to its short effective pathlength, makes it unsuitable as an alternative to transmission-based techniques in applications that require the measurement of components present in oils at low concentrations, such as the determination of peroxide value or free fatty acid content.

Although additional intra- and interlaboratory validation studies are required to assess the robustness of the IV method developed in this work, we suggest that the principles of this method can serve as the basis of an official method for the determination of IV by FTIR/SB-HATR spectroscopy. Apart from the benefits of speed, simplicity, and amenability to automation associated with FTIR analysis (1), such a method would have the advantage of utilizing the same instrument/sample-handling configuration as the recently adopted AOCS SB-HATR method for the determination of *trans* isomers. Because FTIR spectra are collected as single-beam spectra and subsequently digitally ratioed against a background spectrum to produce absorbance spectra, the use of different background spectra in the PLS-based IV method (open-beam spectrum as background) and the official *trans* method (reference oil spectrum as background) does not preclude using the same single-beam spectrum of the sample for both determinations, as was done in the present work. Accordingly, an analytical package could be developed for the simultaneous determination of *trans* content and IV, utilizing

the presently accepted official *trans* method and a "universal" PLS-based IV calibration. With the availability of such a package, the FTIR/SB-HATR technique could be readily implemented in QC laboratories and would provide a rapid, simple, and cost-effective alternative to GC analysis in situations in which only IV and *trans* data are required.

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CHAPTER 8

SUMMARY AND CONCLUSION

The research described in this thesis was undertaken to explore applications of quantitative FTIR analysis in the quality control of fats and oils. The first aspect addressed in this work was the potential utility of FTIR spectroscopy in the assessment of oxidative status and stability. A sound understanding of the spectral changes that take place as an oil oxidizes is a prerequisite to using FTIR spectroscopy to assess the levels of various end products of lipid oxidation. Thus, a detailed examination of the FTIR spectra of oils undergoing oxidation under various conditions was conducted in order to lay the foundation for the development of FTIR methods for assessing oil quality in relation to lipid oxidation and thermal stress. As described in Chapter 3, this study demonstrated that FTIR spectroscopy provides a simple and rapid means of following complex changes taking place as lipids oxidize. Absorption bands associated with common oxidation end products were identified by relating them to those of spectroscopically representative reference compounds, and "real-time oxidation plots" were generated to graphically represent the time course of autoxidation. The results of this research suggested possible new applications of FTIR spectroscopy for the assessment of oxidative status based on its capability to detect both primary and secondary oxidation products in oils and to track oxidative changes in oils undergoing thermal stress (e.g., frying oils). In subsequent work by the McGill IR Group, quantitative methods for the determination of PV and AV were developed based on the calibration approach proposed in Chapter 3. The experimental protocols for real-time monitoring of

oil oxidation described in this chapter could also potentially serve as the basis for an FTIR oil stability test to act as an alternative to the AOM, the OSI, or the oven test. As discussed in Chapter 2, the relevance of the results provided by the AOM and OSI has been questioned because of the elevated temperatures employed to accelerate oxidation, whereas the oven test, which is conducted at a moderate temperature (60°C), has the drawback of being fairly lengthy. It has been suggested in the literature that the optimal test conditions would involve combining increased access of oxygen with the moderate temperatures employed in the oven test (1). In principle, these conditions would readily be met in an FTIR stability test employing a heated multiple-reflection HATR accessory, since a high surface area/volume ratio can be obtained by spreading a thin film of the oil on the surface of the ATR crystal. The development of such a test would involve establishing a standardized protocol that specifies temperature, surface area, and sample thickness and defining an endpoint in terms of the intensity of a selected band that serves to gauge the length of the induction period and hence the relative stability of an oil.

Following the completion of this first study, which was based on tracking oxidative changes by using differential spectroscopy, the focus of the research shifted to the development of quantitative methods for the bulk characterization of fats and oils based on the application of both univariate and multivariate calibration techniques. In Chapter 4, the development of a rapid FTIR method for the simultaneous determination of *cis* and *trans* content, IV, and SN was reported. The PLS calibration models employed in this method were developed with the use of pure triglycerides as calibration standards, with the aim of obtaining "universal" calibrations that would be applicable to any refined triglyceride-based oil. As reported in Chapters 4 and 6, the validity of this calibration

approach is indicated by the good agreement obtained between FTIR-predicted and GC-derived IVs for oils and fats of a wide variety of types.

Chapter 5 addressed the issue of peak height *trans* analysis. This work was carried out in the context of modifications of the traditional IR method by other workers as well as the need to find a means of validating the PLS *trans* calibration model reported in Chapter 4. The practice of spectral ratioing against a *trans*-free reference oil, employed in an ATR method reported in the literature, was adopted in the development of a peak-height-based transmission method. Both the PLS and the peak height *trans* calibration were validated by analyzing Smalley check samples, and the two calibrations produced similar results. Whereas the peak height method employs spectral ratioing to eliminate the underlying absorptions that limit the accuracy of the traditional IR *trans* method, the PLS-based *trans* method mathematically compensates for them within the calibration model. The excellent correspondence between the results from the two methods provides indirect experimental evidence for the validity of the two different approaches employed to compensate for underlying absorptions. There is, however, a fundamental difference between these approaches, which is of some significance. Whereas the accuracy of the peak height method is dependent on the use of a *trans*-free reference oil whose triglyceride composition closely matches that of the sample, the PLS method is universally applicable and eliminates this requirement, which can be problematic to meet.

Chapter 7 considered the utilization of the SB-HATR sample-handling technique for the determination of *trans* content and IV via a PLS calibration approach by applying the same principles as employed in the development of the transmission method described in Chapter 4. This work was undertaken because the use of the SB-HATR

technique, in combination with the spectral ratioing principle described in Chapter 5, had been deemed an AOCS Recommended Practice for the determination of isolated *trans* isomers based on *trans* peak area measurements. The SB-HATR technique was shown for the first time to be suitable for the determination of IV in addition to *trans* content. A significant aspect of this finding is that the IV method is based on a multivariate calibration and hence, as discussed below, imposes more stringent requirements on spectral quality and reproducibility than the univariate *trans* peak area method. In this study, as in Chapter 6, the results obtained by the PLS *trans* method were in good agreement with those of the peak area method. Good agreement was also found between the FTIR *trans* predictions and *trans* data obtained by a GC method that is optimized to quantify *trans* fatty acids, which has recently been approved by the AOCS. This finding corroborates reports in the literature that the recent modifications of the AOCS IR and GC methods appear to have eliminated the traditional discrepancies between IR and GC *trans* results, indicating that accurate *trans* values can now be obtained by either method, with the caveat that the IR method only accurately measures *trans*-monoenes.

The five papers presented in this thesis, as well as other publications in the literature, have demonstrated the potential utility of FTIR spectroscopy as an analytical tool in the quality control of fats and oils. As exemplified by the IV/SN/*cis*/*trans* method described in Chapter 4, an FTIR spectrometer can, in effect, perform a number of common fat and oil analyses on the basis of a single spectral measurement. Thus, FTIR analysis can provide substantial savings, in terms of time and labor, for quality control laboratories in the fats and oils industry, with the additional benefit of reducing the use of solvents and chemical reagents. However, FTIR spectroscopy can only realize its

potential as a practical process and quality control tool if it can be demonstrated to be a simple, convenient, and reliable technique. Accordingly, beyond the development and validation of new analytical methods, the work described in this thesis has addressed the need for convenient sample-handling techniques that are suited to an industrial QC environment, the evaluation of long-term calibration stability, the feasibility of transferring calibrations from instrument to instrument, and the packaging of FTIR analytical methods to facilitate their implementation. These aspects of the research are summarized briefly in the following paragraphs.

Sample handling. During the course of prior work in our laboratory on edible oil analysis by FTIR spectroscopy, it had become apparent that the commercially available FTIR sample-handling accessories were not particularly well suited for routine analysis of fats and oils in an industrial setting. For this reason, a heated transmission flow cell accessory meeting the requirements for rapid, semiautomated analysis of fats and oils was designed; schematic drawings of this accessory were presented in Chapter 4. This accessory was employed for the development of the IV/SN/cis/*trans* method and the *trans* peak height method reported in Chapters 4 and 5, respectively, and the validation studies of these methods described in Chapter 6 served to test the reliability of the accessory. In the case of ATR methods, heated SB-HATR accessories have recently become commercially available, and the work reported in Chapter 7 demonstrated that the analytical performance achieved with the use of this type of accessory was comparable to that obtained with the transmission flow cell accessory.

Calibration stability/transfer. The interrelated issues of calibration stability and calibration transfer have not received much attention in mid-IR spectroscopy, largely

because mid-IR analytical methods have traditionally been based on univariate (i.e., Beer's law) calibration equations. These are usually derived with a small set of calibration standards, making it a relatively simple matter to perform a recalibration on a regular (e.g., daily) basis. However, for a variety of reasons, the feasibility of maintaining the predictive accuracy of a calibration over time and transferring a calibration model between instruments becomes more important when multivariate calibration techniques, such as PCR and PLS, are employed. First, the need for recalibration is more problematic in multivariate calibration than in univariate calibration because of the larger number of calibration standards and the greater amounts of time, effort, and expertise usually required. Second, because multivariate calibrations normally use much more of the available spectral information than simple univariate calibrations, they are more susceptible to minor spectral perturbations due to changes in the performance of the instrument or the characteristics of the sample-handling accessory over time (2). Similarly, seemingly minor differences in response between two instruments can have a major effect on the predictive accuracy of the calibration (3), and this imposes severe limitations on calibration transfer.

The key to maintaining the predictive accuracy of a calibration is to understand and control the factors affecting calibration stability. In FTIR spectroscopy, many potential instrumental sources of calibration instability are well controlled. Wavelength precision is maintained because of the use of a laser reference in FTIR spectrometers, thereby virtually eliminating the possibility of wavelength drifts over time, a problem which plagued dispersive instruments. Although there are a number of other possible causes of instrumental drift (e.g., changes in the alignment of the optics, temperature

fluctuations), most of these are compensated for when the single-beam FTIR spectrum of a sample is ratioed against a background spectrum to produce the absorbance spectrum of the sample. The results of the validation studies reported in Chapter 6 provide evidence of good long-term calibration stability because the spectra of the validation samples were collected more than two years after the spectra employed in the development of the PLS calibration models were recorded. An automated calibration update routine was written to compensate for the slight differences in pathlength between the cells used to collect the calibration and validation spectra. This routine has also been used in our laboratory to transfer calibrations between instruments of the same model, and the successful transfer of a univariate *trans* calibration between two different models of spectrometers equipped with cells of different pathlengths was achieved in the study reported in Chapter 5. It remains to be ascertained whether comparable calibration stability and transferability can be attained with SB-HATR methods for the determination of IV and *trans* content.

There is an additional issue affecting the reliability of the predictions obtained from PLS calibration models that has not been specifically addressed in this thesis. When implementing a PLS calibration, it must be recognized that the predicted values for samples whose spectra differ significantly from those of the calibration standards within the spectral region(s) employed for prediction cannot be considered reliable. For example, the IV/SN/*cis*/*trans* method, which is based on calibration models derived using the spectra of pure triglycerides, may not yield accurate predictions for oil samples that contain free fatty acids or are extensively oxidized. Accordingly, any practical implementation of a PLS calibration model should ideally incorporate one or more criteria for flagging "outliers", i.e., samples that are not well represented spectrally by the

standards used to derive the calibration. Means by which such outlier detection can be performed by PLS have been described in the literature (4, 5) and should ultimately be part of any PLS-based analytical method.

Packaging of FTIR analytical methods to facilitate implementation. Because PLS-based FTIR methods are not as straightforward to implement as methods based on a univariate calibration, such as the AOCS isolated *trans* FTIR method, they are not likely to find widespread application unless steps are taken to facilitate their implementation. The optimal approach is the development of analytical packages that allow precalibrated methods to be implemented directly. Ideally, such analytical packages should incorporate the following elements:

- Built-in calibration(s) for a particular analysis.
- Operating software that converts a general-purpose FTIR spectrometer into an edible oil analyzer dedicated to a particular analysis.
- An analytical protocol defining the sample-handling accessory, data acquisition parameters, operating conditions (temperature, time, pathlength, etc.).
- System check and outlier detection routines so as to ensure that analytical performance is not compromised.
- Automatic data processing, output/storage and data management capabilities.

The development of application-specific software is an essential step in packaging FTIR analytical methods. In this regard, one of the major advances in FTIR spectroscopy during the past decade has been the switch to the use of PC-type computers to drive the spectrometer, whereas formerly a dedicated computer, having its own particular (often manufacturer-specific) operating system, was an integral component of all FTIR systems. This has allowed for dynamic data exchange (DDE) between the FTIR software and other

programs operating under Microsoft® Windows. For example, as part of the work reported in this thesis, a program written in Microsoft's Visual Basic programming language was incorporated into an edible oil analysis package to automate the collection of spectra as well as data processing and archiving. The program included several check routines and a pathlength correction routine to ensure that the predictive accuracy of the calibrations was maintained. This program has been extensively tested, not only in our laboratory, but also in an industrial QC laboratory, and was employed in the validation studies presented in Chapter 6

The virtual necessity of packaging PLS-based FTIR analytical methods in the manner outlined above represents an impediment to their acceptance by the industry. For the purposes of setting product specifications and complying with regulatory requirements, the industry relies upon "official" methods of associations such as the AOCS or AOAC. Traditionally, the methods approved by these organizations have been "prescriptive"; that is, the write-up of the method describes the analytical concept, specifies the reagents, and contains a set of step-by-step instructions, thereby providing all the information required to perform the analysis. However, as analytical methodologies become more sophisticated and increasingly involve proprietary elements (e.g., a patented reagent or concept, a proprietary calibration, etc.), many new methods are not amenable to this prescriptive approach, making it necessary for the methodology approval process to focus on performance-based validation. Although this approach has been in place for some time at the AOAC for instrumental methods, as exemplified by IR milk analysis, the AOCS has not taken this approach to date. However, the AOCS has in

place the basic elements of an excellent performance-based validation mechanism in the form of the Laboratory Proficiency (Smalley) Program. Although the focus of this program is laboratory certification, samples analyzed within it are presently available as secondary reference materials, and this program could also readily be expanded to serve a method validation role. The analysis of the Smalley samples in numerous laboratories yields excellent reference values, together with standard deviations that provide an indication of the performance characteristics of the AOCS official methods. It thus provides realistic analytical specifications that can be used as criteria for approval of new FTIR methods. Using this type of validation approach, the approval of an FTIR method would be based solely on its performance, rather than the analytical approach employed or its amenability to fit within the confines of a "prescriptive" method.

In conclusion, a substantial body of literature has accumulated over the past decade indicating that a range of common AOCS methods can be implemented as FTIR procedures. The research described in this thesis represents a fundamental contribution to this evolutionary development of FTIR oil analysis methodology. Although great strides have been made, the routine use of FTIR-based methods by industry is not yet a reality. It may be anticipated that the recent adoption by the AOCS of the FTIR/SB-HATR *trans* method, in conjunction with pending FDA *trans* labeling regulations, will give impetus to industrial consideration of FTIR spectroscopy as an analytical option. The simple, rapid, and solvent-free *trans* method, although not fully illustrative of the powerful analytical capabilities available with the application of more sophisticated chemometric approaches, will, nevertheless, serve to demonstrate to the industry many of the benefits of FTIR analysis. Based on the extensive experience gained during the course of the work

described in this thesis, it is the author's belief that FTIR spectroscopy will ultimately become a common means of fats and oils analysis, although it may be nutrition labeling legislation rather than the merits of the science and technology that opens the door to its industrial implementation (6).

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